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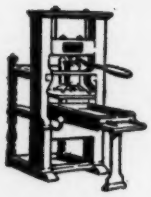

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PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

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PHYTOPATHOLOGY

VOLUME 22 JULY, 1932 NUMBER 7

THE EFFECT OF DIFFERENT TEMPERATURES ON THE REACTION OF HOPE WHEAT TO BUNT[1]

WILLIAM K. SMITH[2]

In a recent paper (17), reference was made to the reaction to bunt of spring-sown and fall-sown Hope (C. I. 8178),[3] a spring variety of *Triticum vulgare* Vill. Because of the possible bearing on the nature of resistance of wheat to bunt, further tests were made on this variety.

Hope has shown consistently a high resistance to various collections of bunt in all spring-sown tests in the hard-red-spring-wheat region of the United States (2, 10, 13). At the Washington Agricultural Experiment Station at Pullman, it has been highly resistant to all collections of the fungus when sown in spring. In the fall of 1928, however, 5 series of 9 winter wheats were inoculated, each series with 1 of 3 physiologic forms of *Tilletia tritici* (Bjerk.) Wint. and 2 forms of *T. levis* Kühn, and planted in the experimental field. Hope was included with the 9 winter wheats in each of the 5 series. A similar set of 5 series of spring wheats, including Hope, was planted in the spring of 1929. The reaction of Hope to the forms of bunt in the 2 tests, along with that of susceptible checks, is shown in table 1. Martin has been included because it differentiates the 3 forms of *T. tritici* and the 2 forms of *T. levis*.

In table 1, the susceptibility of Hope wheat when planted in the fall is in marked contrast with the strong resistance when planted in the spring. A similar situation was observed by Gaines (5) in Marquis, one of the parents of the Hope variety. He suggests that the resistance of Marquis is neutralized by the lowered temperature or the winter rest period. Other spring wheats, such as Florence (C. I. 4740) and Golden Ball (C. I. 6227), which are resistant to Tl and have been tested from fall and spring planting with this form, have been markedly resistant in all tests at the Washington Agricultural Experiment Station.

A comparison of the reactions of fall-sown Hope and Hybrid 128 shows that Hope has, with all 5 forms, lower percentages of bunt than Hybrid

[1] Published as Scientific Paper No. 213, College of Agriculture and Experiment Station, State College of Washington.

[2] The writer is indebted to Dr. E. F. Gaines for the materials used and to Dr. H. F. Clements for advice and criticism in this study.

[3] Accession number of the Division of Cereal Crops and Diseases, U. S. Department of Agriculture.

TABLE 1.—*The reaction of Hope wheat to physiologic forms of bunt when planted in the fall of 1928 and the spring of 1929. Martin differentiates the 3 forms of Tilletia tritici and the 2 forms of T. levis*

Variety	Date of planting	Physiologic forms and percentage of bunt				
		T1[a]	T2	T3	L4[a]	L5
	1928					
Martin	Oct. 9	0	19	71	51	0
Hope	"	51	60	42	61	78
Hybrid 128	"	68	83	90	84	90
	1929					
Hope	Apr. 24	0	0	0	0	0
Jenkin	"	92	78	73	58	56

[a] T and L denote physiologic forms of *T. tritici* and *T. levis*, respectively.

128. Other trials involving the 2 varieties indicate that Hope, when sown at this season, is not completely susceptible. It is, however, apparent that there is a striking difference in Hope between the percentages of bunt in the plantings made on the 2 dates listed in table 1. The greatest contrast between the environmental conditions prevailing in the 2 plantings lies in the trend of temperatures subsequent to the emergence of the seedlings from the soil; in the fall the mean daily temperatures gradually become lower, reaching a minimum towards midwinter, while in the spring they tend to rise rather rapidly. Accordingly, an attempt was made to obtain further information on the relation of the temperature after emergence to the incidence of bunt in this variety.

WORK OF PREVIOUS INVESTIGATORS

Of the many environmental factors influencing the amount of bunt in any variety, temperature is one of the most important. All the available evidence indicates that *Tilletia levis* and *T. tritici* are similar in their reaction to temperature.

Earlier work on the relationship between temperature and infection has been reviewed adequately by Woolman and Humphrey (21) and Caspar (3). It is sufficient here to note that, although the earlier investigators were not in agreement as to the optimum temperature for infection, the situation has now been clarified by studies on the temperature range for the germination of wheat seeds and bunt spores. The data given by Hecke (8), listed below, are in general agreement with those of other workers.

A comparison of the data on the germination of wheat seeds and bunt spores indicates that a certain temperature would be optimum for the in-

Germination temperatures

	Wheat	Bunt spores
Minimum	3–4.5° C.	slightly less than 5° C.
Optimum	25° C.	16–18° C.
Maximum	30–32° C.	less than 25° C.

fection of inoculated wheat, while, with temperatures rising or falling from this point, there would be a decrease in the amount of bunt. This has been confirmed by Munerati (15), who obtained no bunt in inoculated wheat grown at a temperature ranging from 2 to 4° C. and none in inoculated wheat at 22 to 25° C., although plants grown at intermediate temperatures were heavily smutted. Heald and Woolman (7) made a series of sowings of bunt-inoculated seed under field conditions from August 24 to November 23 in 1914 and obtained low percentages of bunt when either high or low temperatures prevailed during the germinating period, while at intermediate temperatures high percentages were recorded. Although it must be borne in mind that in this test, as in other experiments on temperature relations under field conditions, other disturbing environmental factors play a part, *e.g.*, moisture, the results are in agreement with the deductions drawn from the above-mentioned data of Hecke. Similar results have been obtained by other investigators.

There is general agreement with the findings of Wolff (19) that the wheat plant is infected usually between the time of germination and that of emergence of the first green leaf from the coleoptile. Woolman and Humphrey (22), however, in 1 experiment transferred to inoculated soil seedlings that had been grown in sterile soil and that showed 1.25 to 1.5 in. of green leaf and obtained 2 bunted plants from a total of 52. Transplanting would, however, cause considerable root injury and would check the normal development of the plants. Milan (14) succeeded in infecting wheat plants at any time during their growing period by pricking the base of the culms with a needle or by breaking off the culms near the roots and applying germinating spores of *Tilletia levis* and *T. tritici*. Bunted plants were also obtained by Bodine and Durrell (1) by injecting into the base of the culms or inserting inside the sheath cultures of *T. levis*.

The influence of environmental conditions prevailing after the emergence of the seedling from the soil or of the first green leaf from the coleoptile has received meager study. Hecke (8) points out that the entrance of the fungus into the tissues of the plant marks only the beginning of the fight between host and parasite and suggests that temperature may affect this reaction by influencing (a) the germination of spores and seeds, (b) the length of the infection stage in young plants, and (c) the possibility of the fungus reaching the growing point.

Attention was called by Faris (4) to the possible influence of growth conditions subsequent to germination on the development of bunt. Two varieties of winter wheat were inoculated with *Tilletia levis* and *T. tritici* and germinated in temperature tanks in the greenhouse. One-half of the seedlings of each variety were then planted in the field and the other half in the greenhouse. There was no evidence that the growth of the host after germination had a marked effect on the development of bunt in the 2 varieties.

Caspar (3) made observations on wheat grown in the shade of fruit trees and in the open to determine the effect of shade on bunt reaction. The presence of more than one variable factor renders the results inconclusive. Rabien (16) suggests that, since partly smutted plants occur, the environment after the germinating period may affect the reaction of plants to bunt.

It is apparent that little consideration has been given to the effect of environmental conditions after the emergence of the seedlings from the soil. On the contrary, it has been generally assumed that there is no influence after this stage of development. For example, in a discussion of equipment and methods of studying the relation of soil temperature to disease in plants, Leukel (12) states: "As infection by many of the cereal diseases such as bunt, for example, occurs only during the seedling stage of the plant, it is not always necessary to grow the plants at different temperatures beyond the stage of the fifth, fourth, or even the first leaf." Although, under normal conditions, infection may not take place after the appearance of the first green leaf, the reaction between host and parasite after this stage in infected plants of certain varieties may not be the same at different temperatures.

METHODS AND EXPERIMENTAL RESULTS

Field experiments. The data presented in table 1 show that Hope, when planted on October 9, 1928, was susceptible to 5 physiologic forms, while a planting made on April 24, 1929, was smut-free or strongly resistant to the same forms. During the fall of 1929 and the spring of 1930, plantings of Hope were made at different dates to find the reaction to bunt when seeded under conditions different from those of the usual dates of fall and spring sowing. Jenkin (C. I. 5177), a spring variety of *Triticum compactum* Host, was used as a control. Unfortunately, fall rains did not commence until late in the season, so that the first planting was not made until October 29. Owing to the low moisture content and low temperature of the soil after this date, the seedlings grew slowly and, in the later plantings, had not appeared aboveground when the soil was frozen and the plot covered with its winter blanket of snow. At each date, 3 rod rows of each

variety were sown, the seeds being first inoculated with T2 and planted at the rate of 75 seeds per row. The dates of planting and percentages of bunt are shown in table 2.

TABLE 2.—*Percentages of bunt in Hope and Jenkin, inoculated with physiologic form T2 and sown at different dates*

Date of planting	Variety	Bunt			Average
		Row 1	Row 2	Row 3	
1929		*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Oct. 29	Hope	48	40	49	45.7
	Jenkin	91	97	98	95.3
Nov. 5	Hope	26	25	28	26.3
	Jenkin	67	53	71	63.7
" 12	Hope	8	17	10	11.7
	Jenkin	48	55	41	48.0
" 19	Hope	4	1	0.7	1.9
	Jenkin	6	12	8	8.7
" 26	Hope	2	6	4	4.0
	Jenkin	5	5	14	8.0
1930					
Mar. 14	Hope	5	11	5	7.0
	Jenkin	72	71	61	68.0
" 29	Hope	3	2	2	2.3
	Jenkin	67	63	57	62.3
Apr. 5	Hope	3	4	3	3.3
	Jenkin	73	71	66	70.0
" 7	Hope	0			
	Jenkin	79			
1929					
Apr. 24	Hope	0			
	Jenkin	78			
1931					
Apr. 11	Hope	0.6			
	Jenkin	66			

In table 2 the amount of bunt in Hope in the planting made on October 29 is quite similar to that recorded in table 1 for normal sowing time in the fall. In the plantings made later in the fall, the percentage of bunt decreases in each of the 2 varieties, the reaction of Hope paralleling that of Jenkin. In the spring, owing to the high moisture content of the soil, seeding could not be done earlier than March 14. In the planting made on this date, however, the amount of bunt in Hope is relatively higher than in the

later spring plantings and in those made at the usual time for spring wheat in other years in which the strong resistance of Hope and high susceptibility of Jenkin are apparent. It is probable that if environmental conditions would permit sowing earlier than March 14, the percentage of bunt in Hope would be higher than the percentages recorded for this variety in the spring of 1930.

GREENHOUSE EXPERIMENTS

Reaction to physiologic form T2. The unsatisfactory nature of field experiments in testing the effect of temperature has been noted previously. Accordingly, in the winter of 1929–30, a test was made in the greenhouse to study further the influence of temperature. Hope and Jenkin were again used. The 2 varieties were planted in 4 flats (17 in. × 34 in. × 3½ in.) in alternate rows, the seeds inoculated with T2 being planted 2 in. apart in rows 2 in. apart and at a depth of 2 in. The soil used was a mixture of 3 parts of Palouse loam to 1 part of sand. The boxes were watered at intervals to maintain what was considered the soil-moisture optimum for the development of the fungus; daylight was supplemented from sunset until 1:00 a. m. by a 100-watt Mazda lamp at a distance of approximately 30 in. directly above each box. The material was grown in 3 sections of a greenhouse in which temperatures were maintained by the manipulation of steam valves and air vents; 1 section was kept warm (approximately 21° C.), another cool (approximately 9° C.), and the third intermediate (approxi-

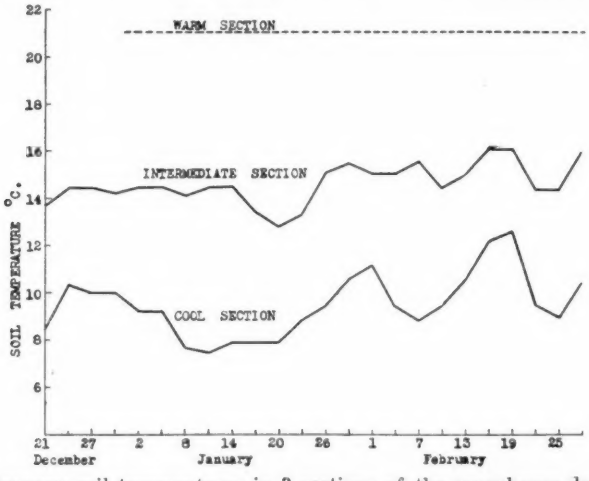

FIG. 1. Average soil temperatures in 3 sections of the greenhouse during a test of the reaction of Hope and Jenkin to a physiologic form of bunt (T2). The curves for the cool and intermediate sections were taken from soil-thermograph records, but the curve for the warm section represents the mean of thermometer readings taken at intervals during each day.

mately 15° C.). Because of the absence of thermostats, there were daily fluctuations of temperature in each section. The variations were brought about by external climatic conditions, so that all sections were influenced in a similar manner. The temperature in the cool and intermediate sections, respectively, was recorded by soil thermographs, while that in the warm section was read at intervals. The trend of temperatures is shown in figure 1, in which the mean temperatures of 3-day periods from December 20 until March 1 are diagrammed.

In figure 1, considerable fluctuation in mean temperature is observed, but the sections in which a continuous record of soil temperature was available show curves that are approximately parallel.

The 2 varieties Hope and Jenkin were planted on December 18 in 4 flats that were treated subsequently as indicated in table 3.

TABLE 3.—*Effect of different temperatures on Hope and Jenkin grown from seeds inoculated with T2 and planted in the greenhouse December 18, 1929*

Temperature during growth	Variety	No. of plants		Bunt	Wt. per plant, excluding roots	Period from planting to heading
		Bunted	Bunt-free			
				Per ct.	*Gm.*	*Days*
Low continuously	Hope	45	0	100	.51	94.3 ± .44
	Jenkin	39	4	90.7	.74	105.6 ± .38
Low until emergence from soil, then intermediate	Hope	18	17	51.4	.26	84.4 ± .54
	Jenkin	35	2	94.6	.31	88.2 ± .56
Intermediate continuously	Hope	17	13	56.7	.27	84.1 ± .74
	Jenkin	33	1	97.1	.37	86.6 ± .59
Low until emergence from soil, then high	Hope	1	40	2.4	.18	81[a]
	Jenkin	37	4	90.2	.16	80[a]

[a] The average number of days from planting to heading in this treatment was computed from counts of headed plants taken at 5-day intervals, whereas in the other treatments the plants, of which the spike had emerged from the sheath, were tagged individually at 2-day intervals.

In table 3 it is apparent that, while Jenkin has a high percentage of bunt at all 4 temperatures, striking differences are present in the reactions of Hope: the amount of bunt in Hope ranges from 100 per cent in the cool section to 2.4 per cent in the flat kept at the low temperature until the emergence of the seedlings from the soil and then transferred to the warm section. It will be noted from the average weight per plant (air-dried) that the plants growing at the lower temperatures were more vigorous than the others. Those grown from emergence to maturity in the warm section

were somewhat stunted in growth, and 25 of the 40 bunt-free plants of Hope were sterile; of the 4 bunt-free plants of Jenkin, 2 were quite sterile and the other 2 produced a few seeds, the remainder of the flowers being sterile. The smutted plants of either Jenkin or Hope in this section showed only rarely any sterile flowers, *i.e.*, if an ovary develops on a spike invaded by the fungus, a smut ball apparently will result. Further, microscopic examination of ovaries from the sterile plants of Hope, treated with suitable stains, did not reveal any trace of the fungus. The above-mentioned 25 sterile plants of Hope and 2 plants of Jenkin may be regarded as smut-free, even though no seeds were produced.

The most striking divergence in the reaction of Hope is the difference between the plants kept continuously at the low temperature and those transferred after emergence to the relatively high temperature. All other important environmental factors, such as moisture and light, were kept as uniform as possible throughout the 3 sections. It may, therefore, be concluded that this difference in the reaction of Hope is due to the difference in temperature after the seedlings appeared aboveground.

It is of some interest to compare the reaction of Hope in the other 2 treatments. Although in the one the plants were kept at a low temperature during what is regarded as the infection stage, the resulting amount of bunt is approximately the same in both, while in the treatment in which the temperature was low continuously the percentage of bunt is much greater. In these comparisons, the controlling action of the temperature after emergence is demonstrated for this variety.

Nature of the reaction. What then are the factors responsible for this reaction in Hope? It has been observed frequently that a susceptible wheat, inoculated at sowing time and germinated at relatively high temperatures, produces at maturity a normal yield of seed and no smut balls. This phenomenon has been explained by assuming that, because of the difference in optimum temperature for the growth of host and parasite, the fungus grows more slowly than the wheat plant and fails to reach the growing points of the shoots. If such were the case in this variety, it might be expected that, at the higher temperatures, Hope would give some evidence of more rapid development than Jenkin. The number of days from planting to heading, presented in table 3, does not support this explanation. It is recognized that this criterion of speed of growth may not be very significant because growth of the culms after the tillering stage takes place mainly through nodal rather than terminal growth; if the fungus reaches the young spike during the tillering period, it will be carried up in the spike as the elongation of the culms takes place. Examination of the data shows that the rate of growth in these varieties, as measured by the time from planting to the emergence of the spike from the sheath, is the reverse

of what might be expected: at the low temperature Hope headed out 11.3 ± .6 days earlier than Jenkin, while at the higher temperature the 2 varieties headed at approximately the same time. Heuser (9), however, points out that one variety may grow more rapidly than other varieties at one stage of growth but more slowly than others at other stages of growth. He cites Criewerer 104, a resistant wheat, which emerged from the coleoptile in a shorter period than other wheats but which headed and ripened later than the others.

In order to test the comparative speed of growth of the 2 varieties in the earlier stages at different temperatures, a test was made in the spring of 1930 similar to that of the previous year. Four flats were again used but the seeds were planted 1 in. apart in the row instead of 2 in. Planting was made on March 16.

It was observed that in all 4 flats the Hope seedlings emerged from the soil somewhat earlier than those of Jenkin. When in any flat the first green leaves had attained an average length of 6 to 7 cm., a measurement was made from the tip of the leaf to the tip of the coleoptile on all seedlings in the flat. The data are summarized in table 4.

TABLE 4.—*Comparative length of the first green leaf measured from the tip of the leaf to the tip of the coleoptile in Hope and Jenkin grown at different temperatures; in each treatment, Jenkin has been represented as 100*

Treatment No.	Temperature during growth	Length of the first green leaf	
		Jenkin	Hope
1	Low continuously	100 ± 1.79	126.4 ± 3.07
2	Low until emergence, then intermediate	100 ± 1.64	124.4 ± 2.45
3	Intermediate continuously	100 ± 1.84	123.9 ± 2.75
4	Low until emergence, then high	100 ± 1.65	118.0 ± 2.14

It is apparent in table 4 that, assuming the growth of the first green leaf to be a criterion of rate of development, Hope grew more rapidly than Jenkin in all 4 flats. Since Jenkin has been represented as 100 in each of the 4 treatments, the growth rates of Hope may be readily compared. The important comparison, however, is between No. 1 and No. 4. If the resistance of Hope in treatment 4 is due to the host outgrowing the fungus at the higher temperature, Hope would be expected to have a greater growth relative to Jenkin in No. 4 than in No. 1. This, however, is not the case. On the contrary, the growth of No. 1 exceeds that of No. 4 by 8.4 ± 3.7. Although this difference is scarcely significant, the data show clearly that the growth of Hope relative to that of Jenkin is not higher when trans-

ferred to high temperature after emergence than it is when low temperature is maintained. Therefore, the high resistance of Hope when transferred to the warm section cannot be attributed to more rapid growth at this stage of development.

DISCUSSION

The results presented in table 3 show that Hope, when grown until emergence from the soil at a temperature considered to be near the optimum for infection and subsequently transferred to a temperature of approximately 21° C., is highly resistant to the T2 physiologic form of bunt. Under field conditions the reaction of Hope to form T2 is almost identical with its reaction to the other 4 forms listed in table 1. It has been shown (17) that the factors for resistance against any 1 of the forms are the same as those for resistance against any of the other 4. It is, therefore, highly probable that the reaction of Hope to these forms at different temperatures in the greenhouse would be the same as the reaction to T2 in this experiment. The striking differences in the amount of bunt in fall-sown and spring-sown plants of this variety under field conditions seem to be due to the fact that in spring planting there is a tendency towards higher temperatures soon after the seedlings emerge from the soil, while in fall planting this rise in temperature does not take place until after an interval of several months.

How, then, is the development of the fungus checked at the higher temperatures? Woolman (20) has shown that at least 1 physiologic form of *Tilletia tritici*, probably identical with T1, enters the tissues of certain resistant and susceptible wheats alike; and there is no evidence that other forms behave differently. We may therefore accept the conclusion of Hecke (8) that the fight between host and parasite only begins after the entry of the fungus into the tissues of the plant.

A number of investigators in Germany and Austria, notably von Tubeuf, Appel and Gassner, von Kirchner, Hecke, and Heuser, have studied the relationship between speed of germination and reaction to bunt. Conflicting results have been obtained; some workers found a distinct relationship, while others found that their results did not allow them to generalize. In the recent investigations of Straib (18), only a low correlation was apparent in 7 spring wheats between reaction to bunt and speed of germination as determined from tests in the seed laboratory. A fairly high negative correlation, however, was found between the amount of bunt in 8 varieties of spring wheat and the average length of the first green leaf a few days after its emergence from the coleoptile. In the present experiment, however, the results indicate that the striking resistance of Hope at the higher temperatures is not due to a relatively greater speed

of growth as determined by growth of the first green leaf or time of heading.

There are numerous references to the relationship between cell-sap acidity and disease resistance in plants, but, as Hurd-Karrer (11) points out, the literature on the question "indicates a wide-spread belief that these are related characters, but leaves the impression that the data on which positive claims are based are too often meager or unconvincing." In 6 resistant and 5 susceptible wheats, Hurd-Karrer found no consistent relationship between resistance to *Tilletia tritici* and titratable acidity or hydrogen-ion concentration. Giljarovskij and Zak (6), however, in a recent study of a number of common and durum wheats report that resistance to *T. tritici* is correlated with energy of growth, together with the change of hydrogen-ion concentration towards the alkaline side and the rise in osmotic pressure.

In recent years, with the increasing number of demonstrations of physiologic forms within species of parasitic organisms, the limited application of the concept of a relationship between gross plant characters and reaction to disease has become increasingly apparent; a host variety may be immune from one physiologic form and quite susceptible to another under the same environmental conditions. The above-mentioned concept may apply to the present example, since in table 1 Hope is equally resistant to all forms when spring sown and susceptible to all forms when fall sown. It is, therefore, possible that at one temperature the internal composition of the variety is favorable for fungal growth, while at other temperatures it may not be suitable. No attempt has been made to obtain information on this point. It is also possible that, in the reaction of Hope to bunt, there is an organization of the protoplasm at the low temperature that permits the development of the fungus, while at the higher temperature the organization inhibits or retards fungal growth.

SUMMARY

Hope, a variety of *Triticum vulgare*, has been highly resistant to 3 physiologic forms of *Tilletia tritici* and 2 forms of *T. levis* when planted at the time usual for spring sowing but has been moderately susceptible to all 5 forms when planted at the usual sowing time in the fall.

Plantings of Hope and Jenkin, a susceptible variety of *Triticum compactum*, were made in the field late in the fall of 1929; the seeds were inoculated with a physiologic form of *Tilletia tritici* (T2) and planted at weekly intervals from October 29 to November 26. The percentage of bunt decreased in both varieties, the reaction of Hope paralleling that of Jenkin.

In the plantings made early in the spring of 1930 Hope was resistant

and Jenkin was susceptible, but in the early plantings Hope was less resistant than when sown at the normal date for spring grains.

The relation between temperature and reaction to bunt at different stages of growth was tested for Hope and Jenkin with seeds inoculated with T2 and planted in the greenhouse in the winter of 1929–30. Hope was resistant when grown at a relatively low temperature until emergence from the soil and then grown at a higher temperature, although plants grown continuously in the cool environment were quite susceptible. Jenkin was quite susceptible under conditions of both low and high temperature.

The different reactions exhibited by Hope in fall and in spring plantings seem to be due mainly to the respective temperatures subsequent to the emergence of the seedlings from the soil.

The relative growth rates of Hope and Jenkin after emergence from the soil showed that Hope is not resistant at the higher temperature by reason of more rapid development as measured by rate of growth of the first green leaf or time of emergence of the spike from the leaf sheath.

It is suggested that the resistance of Hope at the higher temperature is dependent on either unfavorable nutritional conditions or an organization of the protoplasm that at this temperature retards or inhibits the growth of the fungus.

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LITERATURE CITED

1. BODINE, E. W., and L. W. DURRELL. Inoculation of wheat with *Tilletia levis* Kühn. Phytopath. **20**: 663–668. 1930.
2. BRENTZEL, W. E., and RALPH W. SMITH. Varietal resistance of spring wheats to bunt. N. Dak. Agr. Exp. Sta. Bul. 231. 1929.
3. CASPAR, R. Über den Einfluss äusserer Faktoren auf den Steinbrandbefall des Weizens. Kühn Arch. **12**: 205–256. 1926.
4. FARIS, J. A. Factors influencing the infection of wheat by *Tilletia tritici* and *Tilletia levis*. Mycologia **16**: 259–282. 1924.
5. GAINES, E. F. Genetics of bunt resistance in wheat. Jour. Agr. Res. **23**: 445–480. 1923.
6. GILJAROVSKIJ, N., and G. ZAK. Die physiologischen Ursachen der Widerstandsfähigkeit des Sommerweizens gegen Steinbrand (*Tilletia tritici* Wint.). Nauchno-Agron. Zhurnal. (Jour. Landw. Wiss.) **7**: 378. 1930. (Russian. Abst. in Fortschr. Landw. **6**: 106–107. 1931).
7. HEALD, F. D., and H. M. WOOLMAN. Bunt or stinking smut of wheat. Wash. Agr. Exp. Sta. Bul. 126. 1915.
8. HECKE, L. Der Einfluss von Sorte und Temperatur auf den Steinbrandbefall. Ztschr. Landw. Versuchsw. Oesterr. **12**: 49–66. 1909.
9. HEUSER, W. Versuche über den Einfluss äusserer Bedingungen auf die Stärke des Steinbrandbefalles des Weizens. Fühling's Landw. Ztg. **71**: 81–99. 1922.

10. HOLTON, C. S. The relation of physiologic specialization in Tilletia to recent epiphytotics of bunt in durum and Marquis wheats. Phytopath. **21**: 687–694. 1931.
11. HURD-KARRER, A. M. Acidity and varietal resistance of wheat to *Tilletia tritici*. Amer. Jour. Bot. **12**: 359–371. 1925.
12. LEUKEL, R. W. Equipment and methods for studying the relation of soil temperature to diseases in plants. Phytopath. **14**: 384–397. 1924.
13. MCFADDEN, E. S. A successful transfer of emmer characters to *vulgare* wheat. Jour. Amer. Soc. Agron. **22**: 1020–1034. 1930.
14. MILAN, A. Contributo allo studio della biologia di *Tilletia tritici* e *Tilletia levis*. Nuovi Ann. Agr. Min. Econ. Naz. Italy **8**: 3–24. 1928. (Abst. in Rev. Appl. Mycol. **8**: 228–229. 1929.)
15. MUNERATI, O. Le basse temperature al momento della germinazione fanno sfuggire il grano all'attacco della carie? Atti R. Accad. Naz. Lincei V. Rend. Cl. Sci. Fis., Mat. e Nat. **32**: 285–289. 1923. [Cited in Rabien (16).]
16. RABIEN, H. Über Keimungs- und Infektionsbedingungen von *Tilletia tritici*. Arb. Biol. Reichsanst. Land u. Forstw. **15**: 297–353. 1927.
17. SMITH, W. K. Inheritance of reaction of wheat to physiologic forms of *Tilletia levis* and *T. tritici*. Jour. Agr. Res. In press.
18. STRAIB, W. Untersuchungen über die Ursache verschiedener Sortenfälligkeit des Weizens gegen Steinbrand. Pflanzenbau **4**: 129–136. 1927.
19. WOLFF, R. Der Brand des Getreides, seine Ursachen und seine Verhütung. 37 pp. Halle. 1873. Inaug.-Diss. [Cited in Caspar (3) as 1874.]
20. WOOLMAN, H. M. Infection phenomena and host reactions caused by *Tilletia tritici* in susceptible and nonsusceptible varieties of wheat. Phytopath. **20**: 637–652. 1930.
21. ————, and H. B. HUMPHREY. Summary of literature on bunt, or stinking smut, of wheat. U. S. Dept. Agr. Bul. 1210. 1924.
22. ————, and ————. Studies in the physiology and control of bunt, or stinking smut, of wheat. U. S. Dept. Agr. Bul. 1239. 1924.

A NONINFECTIOUS LEAF-DEFORMING PRINCIPLE FROM MOSAIC TOMATO PLANTS[1,2]

H. R. Kraybill, P. H. Brewer, R. W. Samson, and M. W. Gardner

By filtration methods Kraybill and Eckerson[3] separated the juice from mosaic tomato plants into 2 fractions, the residue that contained the mosaic virus and the filtrate that, when introduced into healthy plants, produced certain of the symptoms of typical mosaic, such as a stunting of the plant and various leaf deformities, including the filiform type but no mottling. It was uncertain whether the leaf-deforming principle of the filtrates was infectious, that is, whether it could be transferred from plant to plant, whether it was formed only in the juice from mosaic-diseased plants, or whether it was formed from the decomposition of some compounds present in healthy as well as diseased plants.

In the studies reported in this paper we have found that the leaf-deforming principle in the filtrates is not infectious and that it also differs from the mosaic virus in that it is not diminished in activity by heating at 126° C. for 2½ hours, a treatment which inactivates the virus. The most active preparations are obtained by permitting the juice of mosaic plants to autolyze or ferment previous to filtration. This suggests the possibility that the active principle of the filtrates may be a decomposition product of the tomato-plant juice and may have no relation to the virus. While negative results are not conclusive proof, in no case were active filtrates obtained from healthy tomato plants.

METHOD OF INOCULATION

Young tomato plants having 3 or 4 leaves developed were used for inoculation. In order to get results with the leaf-deforming principle, it is essential to use young plants and to inoculate heavily. In the process of inoculation the leaf was supported with a piece of tissue toweling held in one hand while a drop of the inoculum was placed on a leaflet by means of a pipette. Then, by means of a sterilized needle, the leaflet was scarified and the drop of inoculum spread on the wounds. Several leaflets of each of 3 or 4 leaves were inoculated on each of 3 successive days.

[1] Contribution from the State Chemist and Botany Departments, Purdue University Agricultural Experiment Station, La Fayette, Ind.

[2] Most of the material in this paper was presented before the Twentieth Annual Meeting of The American Phytopathological Society, New York, December 28, 1928, to December 31, 1928. Abstract in Phytopathology, **19**: 108. 1929.

[3] Kraybill, H. R., and S. H. Eckerson. Tomato mosaic. Filtration and inoculation experiments. Amer. Jour. Bot. **14**: 487–495. 1927.

PREPARATIONS USED IN INOCULATIONS

A. A large number of healthy tomato plants were ground in a Nixtamal mill on May 12, 1927. The juice was pressed out in a tincture press. Five hundred cc. of the juice was placed in an Erlenmeyer flask and allowed to ferment for about 2 weeks at room temperature. The material was then filtered through a Jena glass-fritted filter $6/< 7$. The filtrate was used for inoculations.

B. Mosaic tomato plants from the greenhouse were ground in a Nixtamal mill on September 6, 1927. The juice was pressed out and 300 cc. was allowed to ferment from September 6 to October 10. Then, the juice was filtered through filter $6/< 7$. The filtrate was used for inoculation.

C. Healthy tomato plants taken from the greenhouse on September 6, 1927, were used. Juice was prepared in the same manner as B.

D. Mosaic-diseased tomato plants from commercial fields were ground in a Nixtamal mill. Fifteen hundred cc. of juice, pressed out by a tincture press, was allowed to ferment from September 9 to 17, 1927. The juice was then filtered through Jena glass-fritted filter $6/< 7$. The filtrate was used for inoculation.

E. Mosaic-diseased plants from the same lot from which D was prepared were ground in a Nixtamal mill. The juice was pressed out in a tincture press and centrifuged in a Sharples supercentrifuge and then stored in the refrigerator.

F. Mosaic-diseased tomato plants from commercial fields were ground in a Nixtamal mill and the juice pressed out with a tincture press September 23, 1927. About 500 cc. of juice was placed in an Erlenmeyer flask, covered with a layer of toluol, and kept in the ice box from September 23 to October 22. The juice was then filtered through filter $6/< 7$ and kept covered with toluol. The filtrate was used for inoculation.

SYMPTOMS PRODUCED BY THE LEAF-DEFORMING PRINCIPLE

Plants inoculated heavily with noninfectious preparations from mosaic plants are temporarily stunted and the young developing leaves show a range of deformation varying from a slight narrowing to an extreme filiform condition of the leaflets, accompanied usually by considerable puckering, curvature, and twisting (Figs. 1 and 2). Portions of the surface of certain of the deformed leaflets may exhibit a dull greyish cast. There is no mottling of the leaflets as in typical mosaic. Furthermore, the leaf-deformity symptoms do not continue to appear on new growth as in mosaic, but the plants soon outgrow the condition and the later-formed leaves are normal.

FIG. 1. Leaf deformities (narrowing of leaflets, filiform tendencies, and curvature) produced by heavy inoculation of young tomato plants with leaf-deforming principle (preparation D after 4 years of storage). A. Fifth and sixth leaves of 1 plant. B. Fifth and sixth leaves of another plant comparable as to age and time of inoculation. Later, normal growth was resumed.

FIG. 2. A. Filiform leaf deformity produced by heavy inoculation of young tomato plant 2 weeks previously with noninfectious preparation D after it had been heated at 96° C. for 10 minutes. B. Dwarfing of the leaf, as compared with C, and slight narrowing of the leaflets produced by inoculation with the leaf-deforming principle. C. Sixth leaf of uninoculated plant of same age as inoculated plant of which B is the 6th leaf.

LEAF DEFORMITIES PRODUCED WITH INFECTIOUS PREPARATION

Marked leaf deformities were produced in 15 plants inoculated with an infectious preparation E, which had been stored in the refrigerator for 4 months, and in all but one of the plants mosaic mottling also occurred (Table 1).

Inoculations made from these plants (Table 2) showed that the mottled plants contained the virus and that the 1 plant showing only leaf deformities did not contain the virus.

Regardless of what viruses may have been present in the original plants from which preparation E was obtained, these results are of especial inter-

est in view of the fact that Mogendorff[4] was able to produce fern-leaf symptoms only by inoculation with the cucumber-mosaic virus, which will not remain infectious more than 3 days *in vitro*[5, 6] and, consequently, could not have been present in this inoculum.

TABLE 1.—*Inoculations with preparations from healthy and mosaic tomato plants*

Inoculum	Source of inoculum	Date of inoc.	Number plants inoc.	Number showing no symptoms	Number with leaf deformities only	Number with mosaic symptoms
D	Mosaic	12–12–27	15	2	13	0
C	Healthy	12–12–27	15	15	0	0
Water		12–12–27	15	15	0	0
A	"	12–16–27	15	15	0	0
F	Mosaic	12–16–27	15	12	3	0
Water		12–16–27	15	15	0	0
A	Healthy	5–25–27	20	20	0	0
E	Mosaic	1–13–28	15	0	1	14
Water		1–13–28	15	15	0	0
A	Healthy	7–23–28	15	15	0	0
B	Mosaic	7–23–28	15	11	4	0
C	Healthy	7–23–28	15	15	0	0
D	Mosaic	7–23–28	15	7	8	0
F	"	7–23–28	15	13	2	0
Water		7–23–28	15	15	0	0
D	"	11–12–31	20	8	12	0

LEAF DEFORMITIES PRODUCED WITH NONINFECTIOUS PREPARATIONS

With preparation D (3 months old), 15 plants were heavily inoculated and marked leaf deformities developed in 13 of the plants and none showed any mosaic mottling (Table 1). In a similar test made when this preparation was 10 months old, 8 out of 15 plants developed the leaf deformities 10 days after inoculation, and in another test, made when it was over 4 years old, 12 out of 20 plants inoculated developed leaf deformities.

With preparation F (3 months old), 15 plants were inoculated and 3 developed leaf deformities. In a similar test made when this preparation was 10 months old, 2 out of 15 plants developed the leaf deformities in 10 days.

With preparation B (10 months old), 15 plants were inoculated and 4 developed leaf deformities. Check inoculations with pure water and with

[4] Mogendorff, N. "Fern-leaf" of tomato. Phytopath. 20: 25–46. 1930.

[5] Doolittle, S. P. The mosaic diseases of cucurbits. U. S. Dept. Agr. Bul. 879. 1920.

[6] Johnson, James. The classification of plant viruses. Wis. Agr. Exp. Sta. Res. Bul. 76. 1927.

the juice from healthy plants (preparations A and C) yielded no leaf deformities.

All of these preparations had undergone a short period of fermentation previous to filtration and storage.

PREPARATION FROM JUICE OF HEALTHY PLANTS NOT ACTIVE

Attemps were made to determine whether it is possible to secure preparations from the juice of healthy tomato plants that would produce symptoms similar to those produced by preparations from the juice of mosaic plants. Neither of the preparations A and C from the juice of healthy tomatoes produced leaf deformities when used as inoculum, as shown in table 1. At the time of the inoculations (July, 1928) preparation A was 14 months old and C was 10 months old and both preparations had undergone preliminary periods of fermentation.

Although these negative results cannot be interpreted as definite proof, they indicate that the principle in the filtrates of the juice from mosaic tomato plants has some connection with the fact that the plants are diseased.

EFFECTS OF INOCULATION WITH LEAF-DEFORMING PRINCIPLE NOT TRANSFERABLE FROM PLANT TO PLANT

In order to determine whether the leaf-deforming principle is infectious, plants showing the leaf deformities produced by inoculating with the leaf-deforming principle were crushed and the juice used to inoculate healthy plants. Ten plants that had been inoculated previously with preparation D and that showed leaf deformities but no typical mottling were selected for the experiment. The individual plants were ground in an iron mortar that had been previously placed in boiling water for 5 min. Fifty cc. of distilled water was added and the extract poured off into a test-tube and preserved by adding a few drops of toluol. The juice from each plant was used to inoculate 5 healthy plants on each of 3 successive days. In no case did the inoculated plants show any symptoms of typical tomato-mosaic or symptoms similar to those produced by the leaf-deforming principle.

In order to check this procedure, 10 tomato plants that had been previously inoculated with juice from mosaic plants (preparation E) were used in the 2nd experiment. Nine of these plants showed typical tomato-mosaic symptoms and marked filiform leaf deformities, while 1 (N 6, Table 2) of the plants showed no mottling but showed symptoms similar to those produced by the leaf-deforming principle.

A water extract of the crushed tissues of each of the 10 plants was prepared in the same way as in the previous experiment. The juice prepared from each individual plant was used to inoculate 8 healthy plants.

In all cases (Table 2) the 8 plants inoculated developed typical symptoms of tomato mosaic with the exception of the 8 plants inoculated with the juice from plant N 6, which had shown no mosaic mottling. These 8 plants showed no symptoms whatever.

These results show very definitely that the leaf-deforming principle is not infectious and cannot be transferred from plant to plant in the same manner as the virus of typical tomato mosaic.

TABLE 2.—*Inoculations with fresh juice from 9 mosaic plants and 1 (N 6) showing only leaf deformities*

Source of inoculum		Number plants inoc.	Number normal	Number with typical mosaic symptoms[a]
Plant number	Symptoms			
N 1	Typical mosaic and leaf deformities	8	0	8
N 2	"	8	0	8
N 3	"	8	0	8
N 4	"	8	0	8
N 5	"	8	0	8
N 6	Leaf deformities but no mottling	8	8	0
N 7	Typical mosaic and leaf deformities	8	0	8
N 8	"	8	0	8
N 9	"	8	0	8
N 10	"	8	0	8

[a] Certain of the plants showing mosaic symptoms also showed leaf deformities.

LEAF-DEFORMING PRINCIPLE COMBINED WITH POTATO VIRUS FAILS TO PRODUCE STREAK

Further evidence that the leaf-deforming principle is not the tomato-mosaic virus was obtained by the failure to produce the streak or double-virus-mosaic disease in any of a set of 10 tomato plants inoculated with the leaf-deforming principle (D) and the virus present in apparently healthy potatoes. Out of a similar set of 10 plants inoculated only with the leaf-deforming principle, 6 developed leaf deformities, and all in another set of 10 inoculated with tomato-mosaic virus and the potato virus developed the streak disease.

Furthermore, the juice from inoculated tomato plants (M 1 to M 10) showing only the leaf deformities, when combined with the potato virus, failed to produce streak, as shown in table 3, whereas the typical streak developed when the tomato-mosaic virus was combined with the potato virus.

TABLE 3.—*Results of inoculation with potato virus and juice from plants showing only leaf-deformity symptoms*

Source of inoculum	First series		Second series	
	Number plants	Number streak	Number plants	Number streak
[a]M 1 + Potato	5	0	15	0
M 2 + "	6	0	15	0
M 3 + "	6	0	15	0
M 4 + "	6	0	15	0
M 5 + "	6	0	15	0
M 6 + "	6	0	15	0
M 7 + "	6	0	15	0
M 8 + "	6	0		
M 9 + "	6	0	15	0
M 10 + "	6	0	15	0
Potato			15	0
Mosaic tomato + potato	10	10	15	13

[a] M 1 to M 10 are plants showing only leaf deformities.

LEAF-DEFORMING PRINCIPLE HEAT STABLE

The leaf-deforming principle is heat stable. It apparently suffers no loss in activity when heated for 2½ hours at 126° C. in an autoclave. Table 4 shows the results of heating the preparation D at various temperatures, and a leaf from 1 of the subsequently inoculated plants is illustrated in figure 2, A.

TABLE 4.—*Effect of temperature on leaf-deforming principle*

Temperature ± 0.2° C.	Time of heating	Number plants inoc.	Plants showing leaf deformities	Plants showing no leaf deformities
° C.				
80.0	10 min.	10	7	3
84.0	10 "	10	10	0
88.0	10 "	10	10	0
92.0	10 "	10	10	0
96.0	10 "	10	9	1
98.6	10 "	10	6	4
126.0	2.5 hrs.	10	10	0

SUMMARY

Filiform leaf deformities closely resembling certain mosaic symptoms were produced on the new growth of young tomato plants by heavy inoculation with preparations obtained from mosaic plants and rendered noninfectious by filtration. Subsequently normal growth was resumed.

Leaf deformities were not produced by inoculation with similarly prepared juices from healthy tomato plants.

The leaf deformities were readily produced with infectious preparations previously stored *in vitro* so long that the possibility of the presence of active cucumber-mosaic virus was eliminated.

When plants showing marked leaf deformities as a result of inoculation with the leaf-deforming principle were crushed and used as inoculum for healthy plants, no leaf deformities resulted. This indicates that the leaf-deforming principle is not transmissible from plant to plant as is a virus, at least in quantities sufficient to produce symptoms, and that it does not increase in the plant in the absence of the virus.

Further evidence that the leaf-deforming principle is not the tomato-mosaic virus was shown by the fact that the inoculation of healthy tomato plants with the leaf-deforming principle combined with the potato virus did not produce the disease known as streak, whereas streak would have been produced had the tomato-mosaic virus been present. Likewise, inoculation with the juice of plants showing only the leaf deformities and with the potato virus failed to produce streak.

The leaf-deforming principle was not destroyed by heating for 2½ hours at 126° C. This indicates that it is not of the nature of a virus and that it is nonliving.

In general, the most active filtrates were prepared by permitting the juice from mosaic plants to autolyze or ferment. This suggests the possibility that the leaf-deforming principle may be formed through decomposition of the constituents of the plant juice and may possibly have no relation to the presence of the virus. However, filtrates similarly prepared from the juice of healthy tomato plants, when inoculated into young, growing tomato plants, failed to produce any leaf-deformity symptoms.

These results indicate that the leaf-deforming principle is produced only in the diseased tomato tissues. Whether the principle is a product of the virus or merely a decomposition product of the constituents of the mosaic plant is uncertain.

A HITHERTO UNREPORTED DISEASE OF MAIZE AND BEANS

W. W. MACKIE

In September, 1929, the writer found what appeared to be a hitherto unreported disease in maize in the experimental plots on the University Farm at Davis. Soon after, the same disease symptoms were noted on common beans, *Phaseolus vulgaris,* in the plots and also on Blackeyes, *Vigna sinensis,* at Hilmar, in the San Joaquin Valley. Specimens sent to Helen Johann, pathologist in the Division of Cereal Crops and Diseases of the United States Department of Agriculture, were identified as *Sclerotium bataticola* Taubenhaus. J. J. Taubenhaus, pathologist of the Texas experiment station, who first discovered and named this fungus in New Jersey in 1913 (15), confirmed the identification.

Appearance of the disease on maize. Charcoal rot, as it was first named by Taubenhaus, is not manifest in the maize plant until the latter approaches maturity. At this time the affected plant shows signs of premature ripening and later may bend or break just above the crown, causing severe lodging. When the stalk is split the pith and fibers appear as though dusted with fine particles of charcoal (Fig. 1, D). These black bodies are small sclerotia, varying from 1/20 to 1/5 mm. in diameter. This condition extends into the roots for an undetermined distance, but at least 12 in. or more. The black sclerotia may be found in the stalk to a height of 8 or 10 in. but have not been found beyond this point. The nodes of the infected area are darkened and frequently show a dark pink discoloration (Fig. 1, D). This color does not always occur and in most cases can be ascribed to the effects of *Fusarium moniliforme,* which is very common in California cornfields. The association of the 2 fungi in the maize plant is common, but apparently association is not necessary for both are found existing one without the other (Fig. 1, A and C).

Within the cornstalk the black sclerotia are found more densely clustered about the vascular bundles to which they appear attached (Fig. 1, E). In the inner medulla or pith, sclerotia appear in fewer numbers. Sclerotia are not found attached to or included in the primary cortex (Fig. 1, D).

The action of the fungus upon the plant is seen in the interference with the passage of water in the xylem, but soon the elaborated materials in the phloem are affected. This combination of injury causes wilting, premature ripening, and shriveled or underdeveloped kernels. At this time the pith disintegrates and the vascular bundles separate into single strands (Fig. 1, A and C). This condition soon leads to the lodging of the plant.

Charcoal rot in beans. The appearance of charcoal rot in bean fields is first seen when the plants are nearly full-grown. At this time a tendency

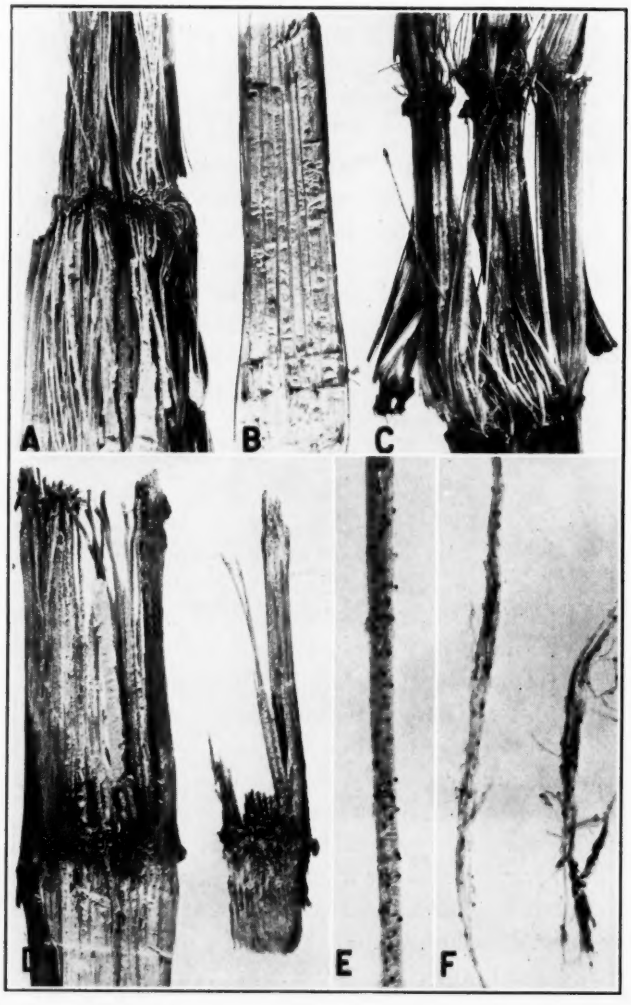

FIG. 1. A–D. Maize stalks. Disintegration of pith and separation of bundles due to *Rhizoctonia bataticola* alone in A and to *Fusarium moniliforme* alone in C. The stalks shown in B are free from disease. Both fungi are present in D. The sclerotia in the internodes belong to Rhizoctonia, but most of the discoloring, which is pink, in the nodes is due to Fusarium. E. Vascular bundle of maize with attached sclerotia. F. Vascular bundles of beans with attached sclerotia.

to wilting, yellowing of the natural, healthy green color, and premature ripening is noticed. Many fields presented the characteristic oil spots that are associated with the spread of a disease from a central point of infestation.

As in the case of maize, charcoal rot in the bean plant is found commonly associated with Fusarium. The Fusarium is readily seen in the pink discolorations, which may or may not occur in plants affected by the charcoal rot alone. Inasmuch as the Fusarium wilt attacks early in the life of the bean host and the charcoal rot appears late, the latter has been classed by some pathologists as a saprophyte (6). The fact remains, however, that the most deadly injury from the charcoal rot appears when the Fusarium wilt is not present.

Upon dissecting the bean plant, the diseased area, as indicated by the presence of the black sclerotia, extends to 8 or 10 in. above the soil, though in Blackeyes a greater height is attained. The roots are affected to an undetermined distance.

The stele is usually found crowded with the black sclerotia, which are attached to the vascular bundles as in maize (Fig. 1, E). In beans, as in maize, the water-carrying vessels appear to suffer the first attack, followed by the injury to the phloem. The wilting in beans is more pronounced than in maize and may induce the farmer to believe that Fusarium is the cause. Premature ripening is very distinct and is seen in the yellowing of the normal green in the leaves, a stiffening or hardening of the stelar structure. Apparently the charcoal-rot mycelium first attacks the water-carrying vessels, for in the stele or woody portion sclerotia are always found, usually in vast numbers, markedly darkening it (Fig. 2). The cambium and meristematic layers are then infected, and here dense clusters of sclerotia may be

FIG. 2. A. Sclerotia on the bast fibers of the common bean, *Phaseolus vulgaris*. B. Black sclerotia beneath the epidermis of the Blackeye cowpea, *Vigna sinensis*.

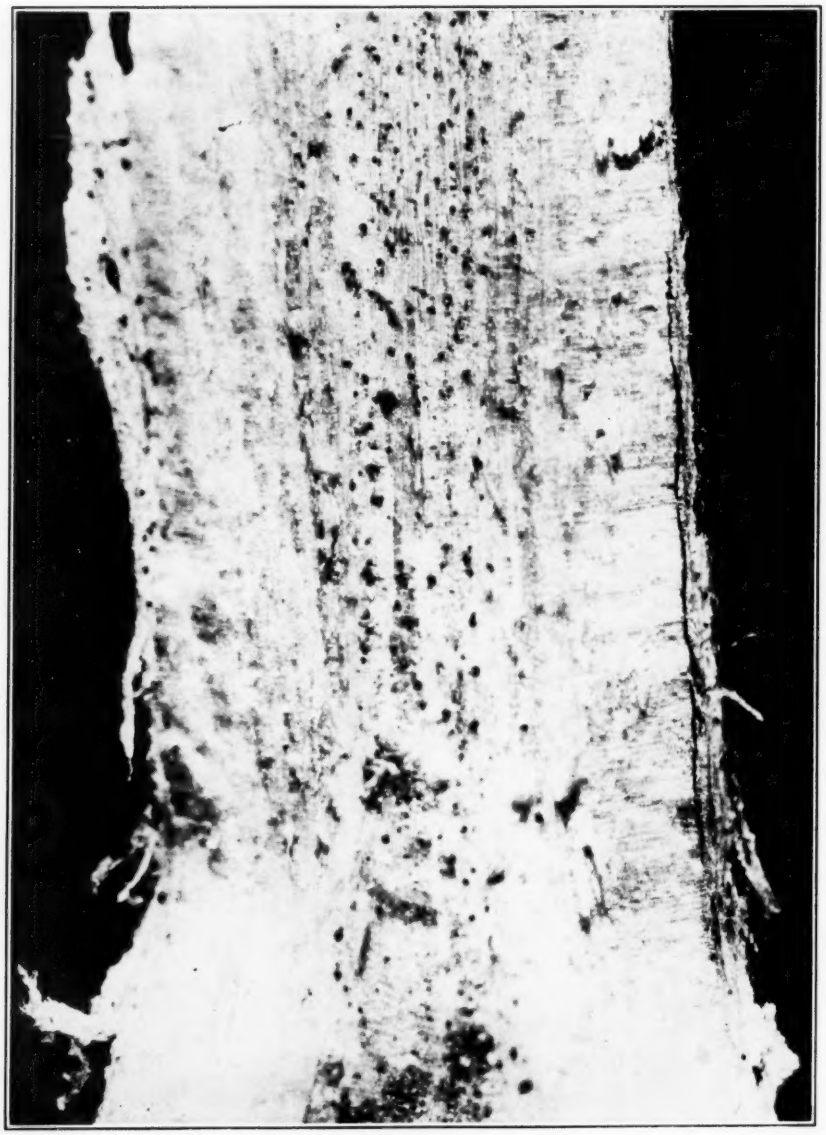

FIG. 3. Bean stem, showing the spread of charcoal rot in the hollow of the stem.

found in the more severely attacked plants (Fig. 2, A). This same condition holds for both roots and stems (Fig. 4). The medulla or pithy portion contains fewer sclerotia than the woody fibers of the state. The sclerotia are attached to the woody fiber of the inner structure and to the bast fibers of the outerbark. The epidermis is not directly attacked, but

FIG. 4. Bean root, *Phaseolus vulgaris*, showing concentration of sclerotia in the fibers just beneath the epidermis.

in extreme cases (Fig. 2, B) the black sclerotia may be seen through the epidermis. The result of this attack is that the diseased plant does not reach its full stature and the seed is reduced in size, corresponding to the completeness of the attack.

Method of infection and spread. Charcoal rot on maize and beans is carried by the sclerotia alone, so far as observed in California. The sclerotia culture readily on several kinds of agar media and within a week abundant sclerotia are formed. There is a general agreement among authorities—Briton-Jones (4), Butler (5), Small (13), and Reichert and Hellinger (10)—that the charcoal-rot-fungus mycelium enters the small feeding roots and travels toward the crown. Martin (8) and Haigh (7) demonstrated that infection is readily induced in the upper portions of certain plants by artificial inoculations in wounds. The rate of progress in beans is considered rapid by Ashby (1), Reichert and Hellinger (10), and others, but in woody perennial plants it may be very slow, according to Small (14), Butler (5), and other East-Indian authorities (11).

The oil-spot spread of the disease observed in bean fields of California indicates that the mycelium may spread through the soil from plant to plant. Laboratory cultures to secure artificial infection are usually not satisfactory. Sclerotia appear to be the only fruiting bodies occurring on beans and corn in California. The disease, therefore, is carried from sclerotia to sclerotia.

The pycnial stage, *Macrophomina phaseoli* (Maubl.) Ashby, found by Ashby (1) and Haigh (7) to occur in beans and sunflowers in Asia, has not yet been found by the writer in California. The sclerotia may remain viable over one or more years, imbedded in the plant tissues or elsewhere. Because of their small size (1/20–1/5 mm.) they may be carried on the seed. It is doubtful if the fungus can be carried internally in the bean seed in the mycelial stage. As the organism apparently does not attack except in the warm summer period, the sclerotia apparently do not germinate except at this time and, hence, overwinter readily.

Hosts. Since 1913, when Taubenhaus (15) discovered charcoal rot in sweet potatoes in New Jersey, the fungus has been found on more than 50 different species hosts wide-spread in the East Indies, West Indies, Rhodesia, Uganda, Egypt, Palestine, India, Formosa, and adjacent Asiatic countries (2, 3, 4, 9, 12, 14). Monocotyledonous and dicotyledonous plants are attacked, annuals and perennials, soft and woody plants in subtropical and tropical areas, principally. The hosts representing crops of interest to the United States include cotton, tobacco, beans, peppers, limes, lemons, oranges, tomatoes, sweet potatoes, cypress, sunflowers, strawberries, roses, and maize. That a single form attacks all these hosts appears unlikely. Haigh (7) has found at least three classes, as indicated by the consistent differences in the size of the sclerotia that occur over several generations.

TAXONOMY

Ashby (1, p. 145) gives synonyms as follows:

Macrophoma Phaseoli Maubl. (1905).
Sclerotium bataticola Taub. (1913).
Macrophoma Corchori Saw. (1916).
Macrophoma Cajani Syd. and Butl. (1916).
Macrophomina philippinensis Petr. (1923).
Rhizoctonia lamellifera Small (1924).
Rhizoctonia bataticola (Taub.) Butl. (1925).
Dothiorella Cajani Syd. and Butl. (1925).
Macrophoma Sesami Saw. (1922).

The form bearing only sclerotia is generally accepted to be *Rhizoctonia bataticola* (Taubenhaus) Butler. The pycnidial form has been identified by Haigh (7) as *Macrophomina Phaseoli* Maublanc. The typical sclerotial form of *R. bataticola* was recovered by Haigh from 50 hosts, but the pycnidial form, from beans and sunflowers only. The pycnidia always give rise to the sclerotia, but the sclerotia do not commonly give rise to pycnidia. The writer has found the sclerotial form only in California.

In maize all subspecies appear susceptible. In the Bay Region, Yolo,

and San Joaquin counties, charcoal rot has been found abundant in maize, but the extent of the area affected is yet unknown.

Charcoal rot has been found in beans from Imperial County in the extreme south of California to Shasta County in the north and in the coastal as well as interior regions. All species of beans grown commercially in California, including *Phaseolus vulgaris, P. multiflorus, P. lunatus sieva,* and *Vigna sinensis,* but excepting *P. lunatus,* were found attacked. Among the common beans, *P. vulgaris,* many varieties, selections, and hybrids appear highly resistant. The Blackeye cowpea, *V. sinensis,* is highly susceptible, but other cowpeas have been found free from attack. The Large Lima, *P. lunatus,* has not been found affected, but one field of Baby Limas, *P. lunatus sieva,* yielded typical diseased plants. The Hopi Limas, which belong to this species, were not observed attacked. All of the few varieties of Butter bean, *P. multiflorus,* under observation were found very susceptible.

SUMMARY AND CONCLUSIONS

1. A fungus disease new to California has been found on maize and beans and the causative organism has been identified as *Rhizoctonia bataticola* (Taubenhaus) Butler.

2. The same fungus has been found on more than 50 plant species.

3. Charcoal rot, the name given the disease caused by the sclerotial form by Taubenhaus, is common in the East Indies and in the subtropical and tropical regions of Asia. It has been found in Egypt, Uganda, and Rhodesia in Africa, the West Indies, and the United States.

4. The pycnidial form is *Macrophomina phaseoli* Maublanc. The pycnidial stage always gives rise to the sclerotial but the sclerotial stage in many cases does not give rise to the pycnidial.

5. Usually, charcoal rot is carried from sclerotia to sclerotia without other spore forms, making it readily separable from most other associated fungi.

6. The fungus attack is made through the young feeding roots in the soil, where it is carried over by the sclerotia. Entrance to older tissue may follow wounds.

7. The only known means for combating this disease in maize and beans lies in breeding for varietal resistance. This is possible as highly resistant varieties have been found.

LITERATURE CITED

1. ASHBY, S. F. *Macrophomina phaseoli* (Maubl.) comb. nov. The pycnidial stage of *Rhizoctonia bataticola* (Taub.) Butl. Trans. Brit. Mycol. Soc. **12**: 141–147. 1927.

2. BRITON-JONES, H. R. On the diseases known as "bark canker" and "die back" in fruit trees. Jour. Pomol. and Hort. Sci. **4**: 162–183. 1925.
3. ————————. Mycological work in Egypt during the period 1920–1922. Min. Agr. Egypt. Tech. and Sci. Serv. Bul. 49. 1925.
4. ————————. West Indies root disease. Trop. Agr. [Trinidad] **5**: 79–82. 1928.
5. BUTLER, E. J. Fungi and disease in plants. 547 pp. Thacker, Spink & Co. Calcutta. 1918.
6. GADD, C. H. Root diseases of economic crops. A reply to Dr. W. Small. Trop. Agr. [Ceylon] **68**: 363–370. 1927.
7. HAIGH, J. C. *Macrophomina phaseoli* (Maubl.) Ashby. The pycnidial stage of *Rhizoctonia bataticola* (Taub.) Butler. Trop. Agr. [Ceylon] **70**: 77–79. 1928.
8. MARTIN, W. H. *Sclerotium bataticola*. The cause of a fruit-rot of peppers. Phytopath. **7**: 308–312. 1917.
9. REICHERT, I. Note sur le Sclerotium parasite du maïs. Rév. Path. Vég. et Ent. Agr. **16**: 230–234. 1929.
10. ————————, and E. HELLINGER. A blight of beans in Palestine. Yedeoth (Proc. Agr. Exp. Sta. Tel-Aviv, Palestine) **2**(½): 61–64. 1929. (Hebrew. English translation on pp. 98–99.)
11. SHAW, F. J. F. Studies in diseases of the jute plant (2). *Macrophomina corchori* Saw. Mem. Dept. Agr. India Bot. Ser. **13**: 193–199. 1924.
12. SMALL, W. A Rhizoctonia causing root disease in Uganda. Trans. Brit. Mycol. Soc. **9**: 152–156. 1924.
13. ————————. *Rhizoctonia bataticola* and root disease. A reply to Dr. C. H. Gadd's criticism of my views. Trop. Agr. [Ceylon] **68**: 370–381. 1927.
14. ————————. On *Rhizoctonia bataticola* (Taub.) Butler as a cause of root disease in the tropics. Trans. Brit. Mycol. Soc. **13**: 40–68. 1928.
15. TAUBENHAUS, J. J. The black rots of the sweet potato. Phytopath. **3**: 159–166. 1913.

THE ADHERENCE OF COPPER DUSTS TO FOLIAGE

L. R. Streeter, E. O. Mader, and F. J. Kokoski

INTRODUCTION

Dusting preparations containing copper sulphate monohydrate ($CuSO_4 \cdot H_2O$) and calcium hydroxide are frequently used as substitutes for Bordeaux mixtures. Many experiments have been reported in which such dusts are compared to Bordeaux mixture for effectiveness in controlling insects and diseases injurious to potato foliage. When copper sulphate monohydrate hydrated lime dust is used, it is assumed that a reaction takes place on the foliage by means of which Bordeaux mixture or some substance with comparable protective properties is formed.

Folsom and Bonde[1] showed by analysis of potato foliage for copper that the copper in Bordeaux mixture adheres better than that in copper-lime dust.

Boyd[2] has shown that, under both greenhouse and field conditions, dusts adhere much better when applied to thoroughly moist leaves than when applied to dry leaves and that dusts applied to moist foliage were more effective in controlling diseases and insect pests than when applied to dry foliage.

EXPERIMENTAL STUDIES

In connection with more recent experiments in the use of copper dusts by the Department of Plant Pathology at Cornell University several experiments have been carried on to determine the effect of moisture and time of application upon the adherence of copper to potato foliage. The dusts were applied with a traction duster to both moist and dry foliage and the conditions noted for each separate test. The preparations used contained 81 parts of hydrated lime and 19 parts of copper sulphate monohydrate. For each treatment 200 gm. of leaves were collected and analyzed for adhering copper. The amount of copper found is expressed in mgm. per 100 gm. of leaves. In each test 2 dust mixtures were used and in this discussion they will be designated as Dusts 1 and 2, respectively.

In the first experiment, July 15, applications were made at 4:30 a. m. and 8:00 a. m. On this date dew appeared at 3:30 a. m. and disappeared at 7:00 a. m. Rain on July 24 amounted to 0.98 in. The results of analyses for copper are given in table 1.

[1] Folsom, Donald, and Reiner Bonde. Potato spraying and dusting experiments 1921 to 1925. Maine Agr. Exp. Sta. Bul. 334. 1926.

[2] Boyd, O. C. The relative efficiency of some copper dusts and sprays in the control of potato diseases and insect pests. N. Y. (Cornell) Agr. Exp. Sta. Bul. 451. 1926.

TABLE 1.—*The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves*

Hour of application	Dust	Date of collection of sample		
		7/15	7/21	7/24
		Milligrams of copper per 100 grams leaves		
4:30 a. m.	Dust No. 1	35	7	trace
	" " 2	30	5	.....
8:00 a. m.	" " 1	22	trace	.....
	" " 2	17	.....	.....

In experiment No. 2 dust applications were made on Aug. 3. At the time of application no dew was present, but a mist-like rain occurred immediately after the dust was applied and the foliage became thoroughly wetted. The rain ceased after 30 min. and, after the foliage became dry, samples of leaves were taken for analysis. Rain on Aug. 3 amounted to 0.40 in. and on Aug. 11 to 0.42 in. The results of sampling on these separate dates are given in table 2.

TABLE 2.—*The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves*

Hour of application	Dust	Date of collection of sample		
		8/3	8/8	8/12
		Milligrams of copper per 100 grams leaves		
8:00 a. m.	Dust No. 1	35	30	7.5
	" " 2	30	22	2.5

In experiment No. 3 applications were made at 12:00 p. m., Aug. 12 and 2:00 a. m., 4:00 a. m., and 6:00 a. m., Aug. 13. Dew appeared at 3:00 a. m. and disappeared at 8:00 a. m. Rain on Aug. 16 amounted to 0.12 in. and on Aug. 22 to 0.54 in. The results of samplings on the 4 different days are given in table 3.

In experiment No. 4 dusts were applied at 8:00 p. m. and 12:00 m. on Aug. 21 and 4:00 a. m. and 8:00 a. m. on Aug. 22. Dew appeared at 4:00 a. m. and disappeared at 11:00 a. m. Rain on Aug. 22 amounted to 0.54 in. and on Aug. 27 to 0.14 in. Results of analyses of samples taken on 3 different days are given in table 4.

In experiment No. 5 dust applications were made at 12:00 m. Aug. 29 and 4:00 a. m. and 8:00 a. m. Aug. 30. Dew appeared at 4:00 a. m.

TABLE 3.—*The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves*

Hour of application	Dust	Date of collection of sample			
		8/13	8/17	8/19	8/22
		Milligrams of copper per 100 grams leaves			
12:00 p. m.	Dust No. 1	34	12.5	trace	0
	" " 2	36	10	"	0
2:00 a. m.	" " 1	39	15	2.5	0
	" " 2	40	10	trace	0
4:00 a. m.	" " 1	45	25	10	trace
	" " 2	41	22	4	0
6:00 a. m.	" " 1	45	27.5	15	2
	" " 2	43	22	8	trace

TABLE 4.—*The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves*

Hour of application	Dust	Date of collection of sample		
		8/22	8/25	8/28
		Milligrams of copper per 100 grams leaves		
8:00 p. m.	Dust No. 1	12	0	0
	" " 2	16	trace	0
12:00 p. m.	" " 1	21	"	0
	" " 2	23	"	0
4:00 a. m.	" " 1	40	10	trace
	" " 2	35	15	"
8:00 a. m.	" " 1	47	35	20
	" " 2	40	30	12.5

and disappeared at 10:30 a. m. No rain occurred during the period of this experiment. The results of analyses of samples taken on these days are given in table 5.

These experiments indicate that the presence of moisture on the foliage is essential to good adhesion of the copper-lime dust, as the adherence of copper is closely correlated with the presence or absence of dew. The writers are of the opinion that the difference in adherence is explained by the fact that the calcium hydroxide is changed to carbonate when moisture is absent, with the result that a good adhering film is not formed.

To test the theory that poor adhesion is due to the conversion of the calcium hydroxide to calcium carbonate, a few laboratory experiments have been performed. A dust was prepared by mixing 20 parts of copper sul-

TABLE 5.—*The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves*

Hour of application	Dust	Date of collection of sample		
		8/30	9/3	9/7
		Milligrams of copper per 100 grams leaves		
12:00 m.	Dust No. 1	25	10	trace
	" " 2	27	9	0
4:00 a. m.	" " 1	45	22	7
	" " 2	40	20	5
8:00 a. m.	" " 1	55	49	15
	" " 2	52	37.5	12.5

phate monohydrate with 80 parts of calcium hydrate. A part of this mixture was exposed for 5 hrs. to air at 70° F. and 80 per cent relative humidity. After exposure this fraction and a part of the original mixture were dusted onto glass plates previously moistened with atomized water. After drying, artificial rain was applied and the adhering copper determined. The results of this experiment are given in table 6. These results

TABLE 6.—*The amount of copper present on glass plates previously moistened with water and then dusted with 20 parts copper sulphate monohydrate and 80 parts calcium hydrate, and subsequently exposed to artificial rain*

Experiment	Original dust	After 5-hr. exposure to air
No.	*Gm.*	*Gm.*
1	0.040	0.022
2	0.033	0.023
3	0.035	0.019
4	0.014	0.005

show further that a certain amount of water of condensation is essential to good adherence at the time of application of the dust.

The above experiments indicate that the change from calcium hydroxide to calcium carbonate may take place in a few hours, but in order to obtain some definite information on the rate of conversion under uniform conditions of temperature and humidity a series of experiments have been carried out in the laboratory.

For these tests high-grade commercial hydrated lime, 99 per cent of which in each case passed through a 325-mesh sieve were used. The hydrated lime powders were dusted onto paper-covered trays and placed in an air-conditioning cabinet. The dust layers were approximately $\frac{1}{3}$ mm. in

thickness. A moderate air circulation was maintained and also a constant change of air from outside the cabinet. The rate of conversion under the several conditions maintained are given graphically in figure 1.

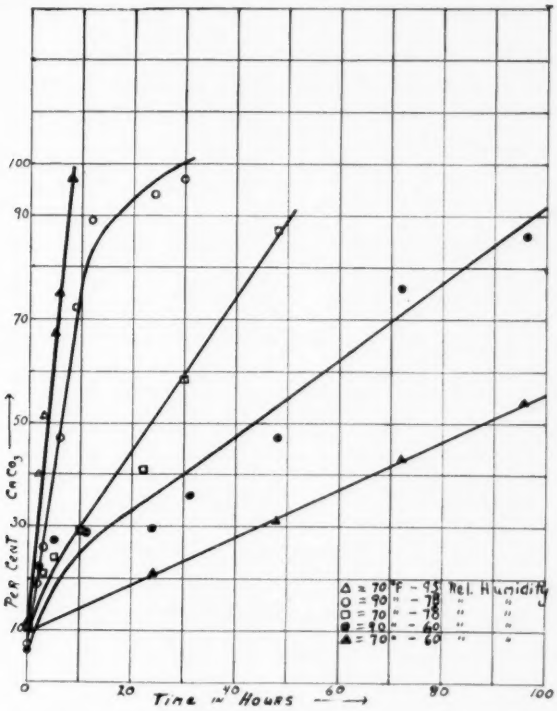

FIG. 1. The effect of temperature and humidity on the rate of carbonation of calcium hydrate.

The conditions of the experiment are not identical with those encountered in the field, but the data give some basis for estimating time during which lime may be expected to function as calcium hydroxide.

These data clearly show that both temperature and humidity are important factors in the conversion of calcium hydroxide to calcium carbonate. It can be concluded further that conditions favoring the conversion of thin layers of hydrated lime to calcium carbonate within a few hours are common in midsummer. These data further emphasize the importance of moisture being present at the time of or soon after the application of dust mixtures, such as copper-lime dusts, from which Bordeaux mixture is supposed to form on the foliage. It is evident that when reactions involving lime take place on foliage, the calcium carbonate as well as the hydroxide content of the lime must be considered.

SUMMARY

The data presented in the foregoing show the importance of timing applications of copper-lime dusts to coincide as closely as possible with the occurrence of water of condensation on the foliage.

It is evident that a high relative humidity increases the rate of carbonation of the hydroxide but does not supply sufficient moisture to convert the dust mixture into a good, adhering film or membrane.

The conversion of calcium hydroxide to calcium carbonate may take place in a few hours or it may take several days, depending on the temperature and relative humidity of the air.

GENEVA AND ITHACA, NEW YORK.

THE EFFECT OF DELAYED PLANTING ON THE CONTROL OF BUNT BY COPPER CARBONATE DUST[1]

H. H. Flor

INTRODUCTION

Many of the popular publications advocating the copper carbonate dust method of seed treatment for control of wheat bunt state, as one of the advantages of this treatment, that it can be done during the slack season and the seed stored for later planting. Heald and Smith[2] showed that treated seed can be stored without injury to germination. However, the writer is unaware of the publication of the results of any test to determine the effect of the lapse of time between seed treatment and planting on the effectiveness of the copper carbonate treatment.

MATERIALS AND METHODS

The effect of planting at weekly intervals following seed treatment was studied at Pullman, Washington, during the season of 1930–31. Seed of Hybrid 128 (C. I. 4512) was inoculated with fresh bunt spores in the proportion of 1 gm. of spores to 100 gm. of wheat. The inoculum was obtained from E. F. Gaines and was composed of equal parts by weight of 3 forms of *Tilletia tritici* (Bjerk.) Wint. and 2 forms of *T. levis* Kühn. Hybrid 128 is susceptible to all of these forms. The inoculated seed was divided into 3 lots and on September 8, 1930, 1 portion was treated with copper carbonate dust, of 50–52 per cent copper content, at the rate of 4 oz. per bu.; the 2nd lot was soaked for 1 hr. in a 1:320 solution of commercial formaldehyde, covered over night, and then dried. The 3rd lot was not treated. On the following day the seed was put up in packets for rod-row planting. All packets were stored in the laboratory at room temperature and taken to the field as needed for planting.

The seed was planted in the agronomy nursery in plots of 3 rod rows. Four plots of each treatment were planted on each date of seeding. At maturity the bunted and bunt-free heads in the center row of each plot were counted. Soil-thermograph records were taken of soil temperature at the depth of planting, which was 2 to 3 in. below the surface. The 1st sowing was made on September 12, 1930, just after the fall rains had started and while the soil was wet and sticky. The 2nd sowing was made

[1] Cooperative investigations between the Washington Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[2] Heald, F. D., and L. J. Smith. The dusting of wheat for bunt or stinking smut. Wash. Agr. Exp. Sta. Bul. 171. 1922.

on September 15, after the soil had dried out for 3 days. Sowings were made every 7th day thereafter until prevented by snow and frozen ground.

RESULTS

The results of this test are given in table 1.

The small amount of bunt in the inoculated check plots of the 1st and last sowings indicates that earlier or later sowings would probably have been of little value, as conditions were unfavorable for bunt infection. Favorable conditions for bunt infection existed during the period from the 3rd sowing on September 22 until the 9th, on November 3, as shown by the high percentage of bunted heads produced in the inoculated plots.

The most interesting feature of this test was the gradual increase of bunt in the plots sown with copper carbonate-treated seed during the period covered by the first 9 sowings, while the formaldehyde treatment was uniformly effective throughout the test. This is brought out more clearly by a comparison of the 2 columns showing the proportional reduction of bunt by the 2 treatments. During the period covered by the first 5 sowings, the copper carbonate and the formaldehyde treatments were about equally effective. The formaldehyde treatment usually gave better control, but the differences were not significant. The gradual increase in percentage of bunt in both treatments during this period may be accounted for by the increasing severity of soil infestation. Heald and Gaines[3] have shown that soil infestation due to wind-blown spores, at Pullman is usually on the increase during this period and reaches its maximum during the 1st week in October.

All sowings subsequent to that of October 6 show a great difference in the effectiveness of the 2 treatments. The formaldehyde treatment varied in efficiency from 92.3 to 100 per cent, when compared with the percentages of bunt from nontreated seed, while the efficiency of the copper carbonate treatment varied from 81.0 to 64.8 per cent. The differences in the effectiveness of the 2 treatments in the last 5 sowings were many times the probable error in every instance and, therefore, were undoubtedly significant. The temperature at the time of the 10th sowing was too cold for good bunt infection, as the plots sown with untreated seed had but 21.3 per cent infected heads as compared with 89.3 per cent in the sowing of the previous week. The copper carbonate treatment in the last sowing reduced bunt to 5.7 per cent, or 25.8 per cent of that in the inoculated nontreated check. This was the only sowing in which the copper carbonate treatment did not show a decrease in effectiveness when compared with the one made the previous week, but, in view of the decrease in bunt in the check

[3] Heald, F. D., and E. F. Gaines. The control of bunt or stinking smut of wheat. Wash. Agr. Exp. Sta. Bul. 241. 1930.

TABLE 1.—*The influence of the date of planting on the effectiveness of copper carbonate and formaldehyde seed treatments for the control of wheat bunt*

[Seed of Hybrid 128 wheat, inoculated with *Tilletia levis* and *T. tritici* and treated September 8, 1930, with 50–52 per cent copper carbonate, 4 oz. per bu., and 1: 320 formaldehyde solution by soaking seed 1 hr. and covering overnight]

Seed sown	Precipitation[a]	Soil temperature[b]			Average percentage of bunted heads from inoculated seed			Proportionate control by	
					Untreated	Treated with—			
		Max.	Min.	Ave.		Copper carbonate	Formaldehyde	Copper carbonate	Formaldehyde
		°F.	°F.	°F.					
Sept. 12	.....	.....	.....	.....	14.6 ± 3.89	0.8 ± 0.24	0.6 ± 0.14	94.5	95.9
" 15	0.25	82	51	66	45.8 ± 2.88	2.7 ± 0.17	3.4 ± 0.68	94.2	92.6
" 22	0.02	79	46	60	87.3 ± 3.00	6.9 ± 0.93	1.9 ± 0.24	92.1	97.8
" 29	0.32	77	46	59	84.3 ± 2.19	10.2 ± 2.20	9.7 ± 0.90	87.3	88.5
Oct. 6	0.23	[c]	.....	.....	93.3 ± 0.88	13.2 ± 1.02	10.0 ± 1.48	85.8	89.3
" 13	0.32	60	34	42	92.9 ± 0.69	17.6 ± 0.96	5.1 ± 1.20	81.0	94.5
" 20	0.40	57	36	45	93.5 ± 1.28	22.3 ± 1.78	4.7 ± 1.23	76.1	95.0
" 27	0.29	60	35	46	93.6 ± 0.54	28.3 ± 1.90	6.3 ± 0.84	69.7	93.3
Nov. 3	0.00	55	36	46	89.3 ± 1.34	31.4 ± 0.53	6.9 ± 0.74	64.8	92.3
" 10	0.14	50	28	35	21.3 ± 1.53	5.7 ± 0.90	0.0 ± 0.0	74.2	100.0

[a] For week ending on dates indicated.

[b] Average for first weeks following seeding, taken at depth of planting.

[c] No data as thermograph failed to record the entire week.

and the perfect control given by the formaldehyde treatment, it is probable that this difference was due to unfavorable environmental conditions for bunt infection rather than to an increase in the toxic action of the copper carbonate.

It is difficult to account for the gradual decrease in efficiency of the copper carbonate dust treatment with each successive sowing over a period of approximately 2 months. There are a number of possible explanations: soil moisture; soil temperature; and a gradual decrease in the toxicity of the copper carbonate when applied to the wheat seed and under the conditions of storage. Unfortunately, soil-moisture records were not taken, but the rainfall records of the experiment station were available. A study of the rainfall records shows that it is doubtful if soil moisture was an important factor. Rains were rather generally distributed over the planting period except for the 9th sowing. No rain had fallen for 9 days prior to or for 6 days subsequent to this sowing, yet there was no difference in the trend of bunt infection. The plots sown with nontreated and formaldehyde-treated seed had approximately the same percentage of bunt as those of the previous sowing, while the plot sown with copper carbonate-treated seed had a small increase in the percentage of bunt. It is possible that soil temperature may have been an important factor in the gradual decrease in the effectiveness of the copper carbonate treatment, for there was a decline from an average of 66° F. during the first week of the periodic sowings to 35° F. during the last week. However, in view of the amount of work that has been done with the copper carbonate-dust treatment by numerous investigators throughout the world, who have found the treatment effective under a wide range of conditions, it is difficult to believe that the production of 31.4 per cent bunted heads in the 9th sowing could be accounted for by the lowered temperature. Further evidence that low soil temperature was not the principal factor in the gradual decreased effectiveness of the copper carbonate treatment is shown by an examination of the soil-temperature records. The maximum, minimum, and average soil temperatures for the weekly periods following the 6th, 7th, 8th, and 9th sowings were essentially similar; yet the percentage of bunted heads in the copper carbonate-treated plots gradually increased from 17.6 per cent in the 6th to 31.4 per cent in the 9th sowing. During this period differences in percentage of bunt in the sowings of inoculated nontreated seed and formaldehyde-treated seed were not significant. Consequently, the most plausible explanation for the relative ineffectiveness of the copper carbonate treatment in the later sowings appears to be the occurrence of something that caused a decrease in the toxicity of the chemical. This decreased toxicity was progressive, becoming more pronounced with the

increased interval between date of treatment and that of sowing. The cause of this change in the toxicity of the copper carbonate, whether physical, chemical or biological has not been studied.

Although the seed used in these tests was smutted much more heavily than seed that would ordinarily be used in commercial plantings and although the test was limited to a 1-year trial, it is thought that the results may explain, to some extent, the numerous failures that have been reported in the commercial use of the copper carbonate-dust treatment.[4] Undoubtedly, many of these cases of failure were caused by inefficient methods of seed treatment, which failed to distribute the dust evenly over the seed. However, the results of this test indicate that some of the failures may have been due to the practice of treating the seed in the slack season and storing until time of sowing, as advocated in many of the popular articles on this method of seed treatment. There is need of a more thorough study of the effect that the interval of time elapsing between seed treatment and sowing has on the effectiveness of the copper carbonate method of treatment for control of bunt.

SUMMARY

The comparative efficiency of the formaldehyde-soak and the copper carbonate-dust treatments was tested by periodic sowings at weekly intervals. The effectiveness of the formaldehyde treatment was fairly constant, varying from 88.5 per cent in the 4th planting to 100 per cent in the 10th. The effectiveness of the copper carbonate treatment decreased with each successive sowing from 94.5 per cent in the 1st to 64.8 per cent in the 9th. Up to and including the 5th weekly sowing, the differences in effectiveness of the 2 treatments were not significant, but in sowings made later the copper carbonate was decidedly inferior.

[4] Haskell, R. J., R. C. Rose, W. E. Brentzel, E. A. Walker, and Waldo Kidder. Why so much smut in spring wheat? U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. Sup. 77: 97–139. 1930. (Mimeographed).

THE BEHAVIOR OF BACILLUS AMYLOVORUS IN SOIL

P. A. Ark[1]

Comparatively few experiments seem to have been made bearing on the relation of *Bacillus amylovorus* (Burr.) Trec. to the soil. Arthur[2] reported in 1886 that the organism grew "with moderate readiness" in soil extract. Waite,[3] in 1895, presented the following summary statement: "It has been claimed that the blight microbe lives over winter in the soil and for a long time the writer supposed this to be the case, but after careful investigation the idea was abandoned for in no instance could it be found there."

The present paper deals with a somewhat specialized technique for the separation of the organism from the soil and the application of this technique to some soils infested by both artificial and natural means.

MATERIALS AND METHODS

The technique employed here diverged from that in general use in bacteriological laboratories in two particulars and is partly an application of Hotson's[4] method for isolating the pear-blight organism. First, in order to inhibit the gram-positive organisms present in large numbers in unsterilized soil, Patel's oxgall medium[5] was used with the following modification: Crystal violet 1:100,000 was used instead of 1:500,000. Second, surface-sterilized green pear fruits or portions of these were punctured with needles and immersed in suspensions of the soils to be tested and then removed and incubated at a temperature favorable for *Bacillus amylovorus*. This not only serves to increase the blight organism to the point where it can be readily isolated by ordinary culture methods but also results directly in symptoms of blight accompanied by the characteristic bacterial exudate. Of 35 cultures of bacteria tested on pear fruits, including 15 species, namely, *Bacterium juglandis* (Pierce) E. F. Sm., *Bact. viridilividum* Brown, *Bact. savastanoi* E. F. Sm., *Bact. phaseoli* E. F. Sm., *Bact. medicaginis* var. *phaseolicola* (Burkh.) Link & Hall, *Aplanobacter michiganense* (E. F. Sm.) E. F. Sm., *Ps. cerasus* Griffin, *Bact. tumefaciens* E. F. Sm. & Town., *Bacillus*

[1] This work was done under the direction of Professor R. E. Smith of the Division of Plant Pathology, University of California.

[2] Arthur, J. C. Pear blight. *In* Report of the botanist. N. Y. (Geneva) Agr. Exp. Sta. Ann. Rpt. **5** (1886): 259–273. 1887.

[3] Waite, M. B. The cause and prevention of pear blight. U. S. Dept. Agr. Yearbook **1895**: 295–300. 1896.

[4] Hotson, J. W. Fire blight on cherries. Phytopath. **5**: 312–316. 1915.

[5] Patel, M. K. An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. Phytopath. **16**: 577. 1926.

carotovorus L. R. Jones, *Bact. aroideae* (Towns.) Stapp, *B. subtilis* (Ehrenb.) Cohn, *Bact. coli* (Esch.) Mig., *B. megatherium* de Bary, *B. faecalis alkaligines* Cast. & Chalm., *Staphylococcus albus* Rosenb., none produced an exudate that need be confused with that of the blight organism. (Fig. 1.) More than 750 uninoculated fruits with and without puncturing were incubated as controls in the course of these experiments, but none of them developed any symptom of blight.

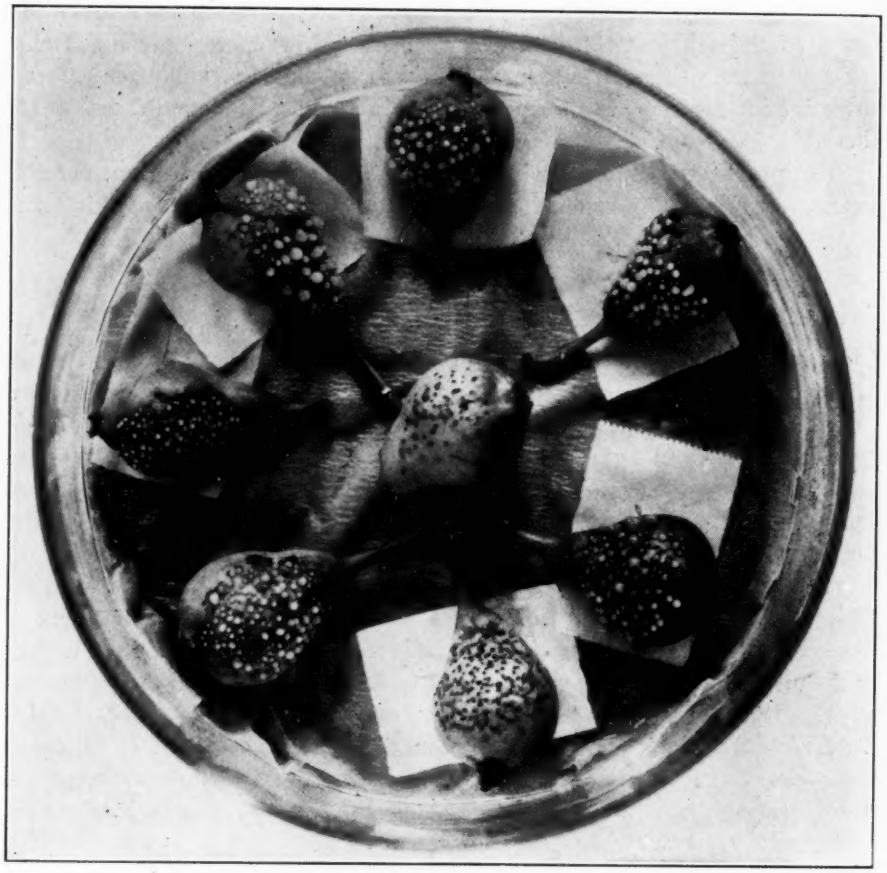

FIG. 1. Whole immature pears with profuse bacterial exudate due to immersion in water suspension of natural soil taken from beneath blighted tree. The pear in the center is the control.

For the preliminary laboratory tests, soils of 3 diverse types were infested: loam surface soil, clay subsoil and sand; the last containing very little organic matter. These were air-dried, passed through a 20-mesh screen, and placed in 150-gm. lots in 300 cc. flasks. Soils were infested by

moistening throughout with beef-peptone-broth cultures of the blight organism. Sterile water and implements were used in suspending and handling the soils. For each soil, 3 flasks were steam-sterilized before sowing with the blight organism and 3 were infested without sterilization. One flask of each lot was incubated at each of the 3 temperatures, 8°, 21°, and 28° C. Both the crystal violet-bile agar and the pear fruits were used in recovering the organism from artificially infested soil.

CULTURES FROM ARTIFICIALLY INFESTED SOIL

Cultures were made from the soils at intervals of 2 or 4 days during a period of 30 days and at long intervals afterward. For the sterilized soils the numbers of organisms per cubic centimeter of soil were computed for each sample. The numbers declined rapidly during the first 2 to 4 days and gradually thereafter. It will be seen in table 1 that the loam and clay were more favorable to the persistence of the organism than the sand.

TABLE 1.—*Longevity (in days) of Bacillus amylovorus in soils at various Centigrade temperatures*

Type of soil	Sterilized			Unsterilized		
	8°	21°	28°	8°	21°	28°
Loam	54	30	30	30	30	30
Clay	54	30	30	38	38	14
Sandy	18	18	0	14	22	18

CULTURES FROM NATURALLY INFESTED SOIL

During the summer, autumn, and early winter of 1931, cultures were made at irregular intervals from soil samples collected beneath blighted pear trees and other susceptible plants. In a few cases samples were taken at some distance from any blight infection. The results are summarized in table 2. The organism was obtained from a considerable number of samples up to the time of the autumn rains.

In several instances the organism was obtained from samples midway between tree rows and in one instance, on November 18, 1931, from an open field from which pear trees had been removed in March or April, 1931.

The soils were first thoroughly wet by rains that fell on November 16, 1931.

The identity of all the organisms was verified by examination in pure culture and all except those of the first lot of samples were tested on shoots of growing potted plants (*Cotoneaster pannosa*) in the greenhouse. For the first lot the organisms were reintroduced into green pear fruits and resulted

in typical symptoms on these. The tests on growing shoots have been limited, and it is probable that others of the cultures indicated in column 3, table 2, will produce infection when tested more extensively.

No relation is apparent thus far between the persistence of the organism and the type of soil (sandy loam to silt loam) or the depth of sampling (down to 5 or 6 in.).

TABLE 2.—*Cultures from naturally infested soils*

Date	No. of samples	Infection on pear fruit	Positive on shoots	Locality
7–8–31 } 7–23–31 }	50	8	Not tested	Alameda County
11–10–31	8[a]	7	7	Sacramento Valley
11–18–31	6[b]	5	4	Napa County
11–18–31	20[c]	14	3	Sacramento Valley
11–18–31	24[d]	21	7	Sonoma County
12–10–31	5	0	Not tested	Santa Clara County
12–18–31	22	0	Not tested	Sacramento Valley

[a] One sample was taken between 3 trees. Positive on fruit and shoot.

[b] One sample was taken between road and trees, 20 ft. from the nearest pear tree. Positive on fruit and shoot.

[c] Four samples were taken from the area where the blighted trees were pulled out in April, 1931. These samples were negative for *Bacillus amylovorus*.

[d] One sample was taken from the bottom of a pit 12 in. deep from which an infected tree had been dug about 3 weeks before. The stump was almost oozing. Positive on pear fruit and shoot of *Cotoneaster pannosa*. One sample from open field was positive for *Bacillus amylovorus*.

SUMMARY

Bacillus amylovorus persisted for 54 days in sterilized soil and for 38 days in unsterilized soil in the laboratory.

The organism was obtained from orchard soils under conditions indicating persistence for at least several weeks in dry or moderately moist soils.

THE PRODUCTION OF BUNT CHLAMYDOSPORES IN THE VEGETATIVE TISSUE OF THE WHEAT PLANT[1]

H. H. Flor

Wheat is subject to attack by 3 smuts: flag smut, loose smut, and bunt or stinking smut. Flag smut, caused by *Urocystis tritici*, Koern. is usually confined to the vegetative tissues, the spores being produced in slightly raised, white or dull gray stripes in the leaves and occasionally in the stem and inflorescence. Loose smut, caused by *Ustilago tritici* (Pers.), Jens., occurs normally in the inflorescence, converting all parts of it, both vegetative and reproductive, into the spore masses and leaving nothing but the bare rachis. Under greenhouse conditions and occasionally in the field, this smut may produce streaks of spores in the leaves. Bunt, or stinking smut caused by *Tilletia levis* Kühn and *T. tritici* (Bjerk.) Wint., usually has been considered to produce spores only in the ovaries of the host plant, thus differing from flag and loose smuts in that it did not produce spores in the vegetative tissues.

During the winter of 1930–1931 the writer inoculated seedlings of Prelude wheat with paired monosporidial cultures of each of the two bunt organisms. These plants were grown in a greenhouse at Arlington Farm, Virginia, maintained at 20° C., and subjected to a long day with artificial illumination. After the plants had headed, the soil was kept rather dry in order to hasten the ripening of infected heads. When the heads were nearly ripe the soil was given a thorough soaking so as to stimulate secondary stooling and see if infected shoots would not be produced by plants whose first heads had escaped infection, because field observations of partially smutted plants had shown the culms first produced were those that usually remained bunt-free. After the soaking, the plants revived and sent out new shoots from the basal nodes. A number of these shoots were dwarfed and deformed, and the leaves appeared to have wart-like smut galls on them (Fig. 1).

Microscopic examination showed that galls filled with chlamydospores of the bunt organisms had been produced in the parenchymatous tissues of both the leaves and stem. Spores produced in plants inoculated with cultures of *Tilletia tritici* had reticulately marked walls, while the walls of spores produced in plants inoculated with cultures of *T. levis* or the interspecific cross were smooth, like those produced in heads in the normal

[1] Cooperative investigations between the Washington Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

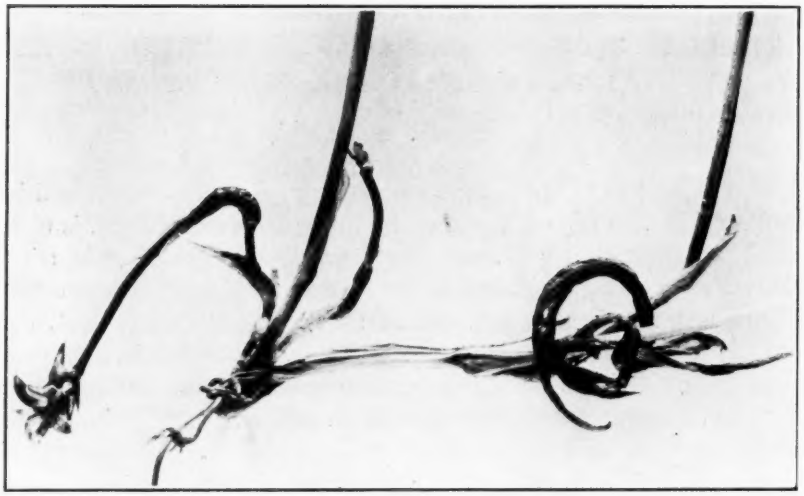

FIG. 1. Base of bunt-infected Prelude wheat plant, showing the secondary culms dwarfed and distorted due to the formation of bunt galls in the vegetative tissues. The leaves on the culm at the extreme left are galled. ×2.

manner.[2] Spores produced in the vegetative tissue were slightly smaller than those in the head of the same culm, being 19.3μ ± 0.097, and 19.9μ ± 0.086, respectively.

A histological study was made of the galls in the leaf and stem tissues. The galls in the leaves occurred in irregular, noncontinuous strands varying from a few millimeters to 1 or 2 cm. in length. They extended from the leaf blade into the undifferentiated leaf and stem tissue at the node. Cross sections showed as many as 4 strands per leaf (Fig. 2, A, C). The strands of bunt spores varied greatly in thickness and diameter, attaining their maximum dimension in the leaf sheath (Fig. 2, B). They always occurred in the parenchymatous areas between or beneath the vascular bundles. Occasionally, fusion had taken place between strands, in which case the vascular bundles had been forced outward. The formation of the gall in the leaf and leaf sheath appeared to be accompanied by a slight hypertrophy (Fig. 2, B, C), but most of the deformity was due to the physical action of the expanding smut gall.

The galls in the stems were inconspicuous and probably would not have been noticed had not the stems been twisted and distorted. In the stems the galls occurred as a single strand made up of a series of swollen pockets connected by narrow necks. As this strand lay in one plane, it caused

[2] Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis*. Jour. Agr. Res. 44: 49–58. 1932.

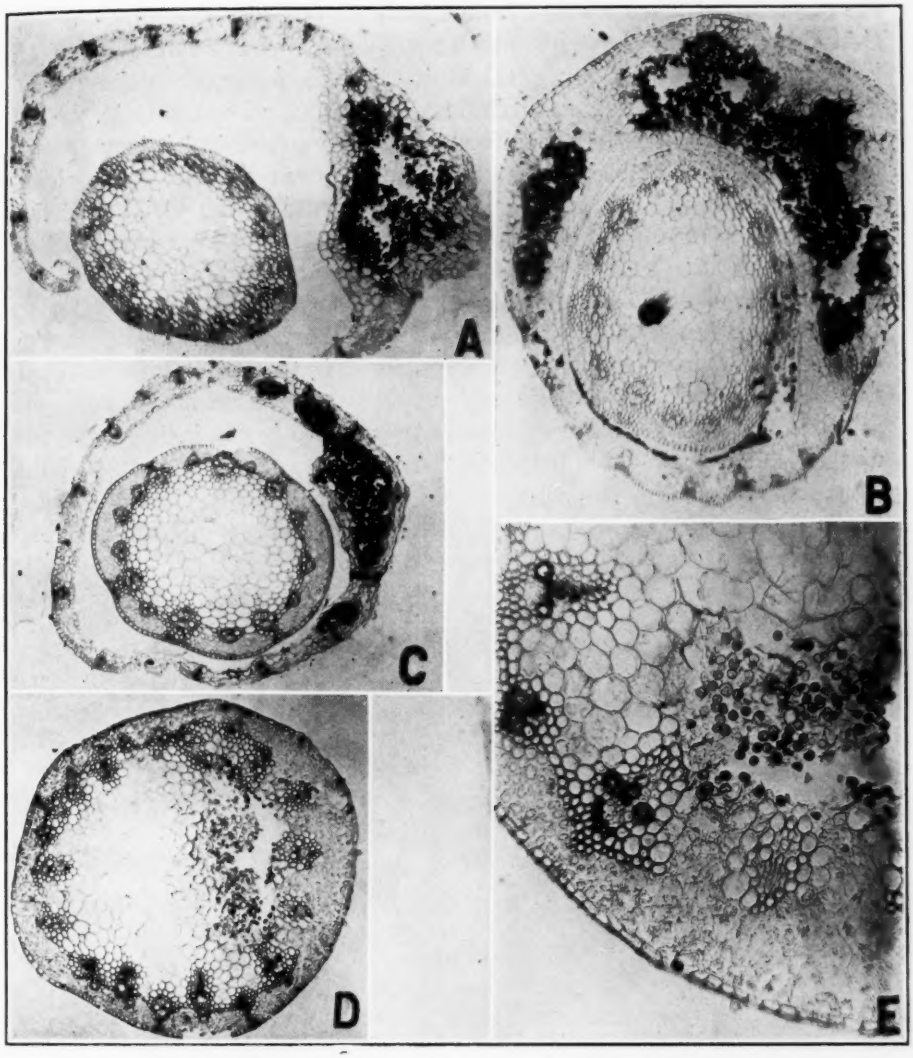

FIG. 2. A. Cross section of a bunt gall in the leaf blade, showing enlargement due to hypertrophy as well as to the mechanical expansion of the gall. ×25. B. Cross section of an infected leaf sheath. The bunt galls extend more than halfway around the sheath, which is enlarged in the galled regions due to the mechanical action of the expanding gall and to the excessive production of parenchymatous tissue. ×25. C. Cross section of a leaf just above the sheath, showing 4 strands of bunt spores in the tissue. ×25. D. Cross section of a galled stem, showing the bunt gall in the pith just beneath the vascular bundles. The cortical parenchyma adjacent to the gall has enlarged and the thick-wall cells that normally connect the vascular bundles and separate the pith from the cortical regions have disappeared. ×25. E. An enlargement of D, showing the inhibitory effect of the bunt gall on the development of thick walls in the conducting and supporting tissues adjacent to the gall and the disorganization of the cortical parenchyma. ×105.

the stem to twist greatly and in one instance to make a complete circle. Gall formation in the stem was accompanied by a small amount of hypertrophy, as shown by a thickening in the parenchymatous region lying adjacent to the gall and outside of the vascular bundles (Fig. 2, D). However, most of the deformity was due to the mechanical injury caused by the expansion of the gall in the tissue, as was the case in the leaf. Another anatomical change was the disappearance in the vicinity of the gall (Fig. 2, D, E) of the thick-wall cells that connect the vascular bundles and separate the central parenchymatous pith cells from the cortical parenchyma.

It is possible that bunt-spore formation in the vegetative tissues of the wheat plant may occur under certain conditions in the field but has not been noticed because of the inconspicuous symptoms. However, it does not seem probable that this is of much economic importance in the incidence of the disease. If these cases of vegetative infection are typical, the symptoms are not likely to be confused with flag or loose smut on leaves and stem because of the tendency of the bunt strands to form spore pockets and the fact that the affected parts are greatly stunted and distorted.

SUMMARY

Chlamydospores of *Tilletia tritici* and *T. levis*, the fungi that cause bunt, were produced in the leaf and stem tissue of Prelude wheat grown in the greenhouse. The affected parts were stunted and distorted.

PHYTOPATHOLOGICAL NOTES

Armillaria Crown Rot of Strawberry.[1] Since the writer first reported a crown rot of strawberry caused by *Armillaria mellea* (Vahl.) Fr. several inquiries concerning its severity have been received, and thus this occasion is taken to report briefly our experience with the disease.

The disease was first observed by the writer several years ago near Albany, Oregon. The infection areas were in the form of 3 almost perfect circles in a Marshall strawberry planting. F. D. Bailey observed a similar case in 1912 near Oregon City (see Fig. 1). These 3 circles were about 7,

FIG. 1. Circular area of strawberries killed by *Armillaria mellea*, near Oregon City, Oregon, June, 1912. Photo by F. D. Bailey.

[1] Published as Technical Paper No. 176 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

11, and 24 ft. in diameter. The planting was examined rather early in April. At this time the plants in the centers of these circular areas had been removed by the grower, having died out during the winter. Plants around the margins of these infection areas were now showing considerable "yellowing" in the mesophyll of the leaves, and the plants were somewhat dwarfed. An examination showed the whole core of the crowns to be almost completely filled with a white fungous felt surrounded by a brownish, crusty layer of plant tissue, perhaps 2 mm. thick. Interspersed in the fungous felt are to be found islands of brownish host tissue. As a rule, rhizomorphs are seldom found around the crowns. Such plants totally collapse early in the summer. The infected plants were about 17 per cent of the total patch. Several instances of this kind have been observed in other locations west of the Cascade Mountains.

In almost every case where the infection areas have been localized, as in circles, the growers have reported that these are the exact spots where oak trees previously stood, and undoubtedly these were the source of the Armillaria.

Two plantings have been studied where infected plants were found singly but well distributed over the whole plantings. These were both plantings of the Marshall strawberry, one on oak-grubbed land the other on land where just previously had been grubbed miscellaneous mixture of brush and shrubby trees of hazel, *Corylus californica* Rose; oak, *Quercus garryana* Dougl.; poison oak, *Rhus diversiloba* Torr. and Gray; dogwood, *Cornus nuttallii* And.; willow, *Salix scouleriana* Barratt; trailing blackberry, *Rubus ursinus* Cham. and Schl.; etc. In the one case of oak-grubbed land there was a great deal of chips and sticks of wood plowed into the soil. Here the strawberry plants infected with Armillaria were found singly over the planting of 5 acres, constituting about 6 per cent loss of plants. In the other case about 23 per cent of the plants of a 7-acre planting showed infection, well distributed over the acreage without definite infection areas.

Notes have been recorded on 6 different plantings of the Marshall variety where Armillaria infections occurred, and 2 others have been observed. These are well distributed in the hill lands of the Willamette Valley, and one infection has been recorded for Clatsop County.

Cultures have been made from 196 plants from which 144 (or 73.5 per cent) isolations of Armillaria have resulted.—S. M. ZELLER, OREGON AGRICULTURAL EXPERIMENT STATION, CORVALLIS, OREGON.

Pineapple Disease (Thielaviopsis Ethaceticus) on Sugar Cane at Tucumán, Argentina. During the latter part of December, 1928, and the first part of January, 1929, the writer cut some P. O. J. 2725 sugar cane from one of the Tucumán Experiment Station plots for the purpose of artificially infesting it with sugar-cane borers (*Diatraea saccharalis* Fab.). In order to do this he made a hole for each borer with a steel punch. He then placed the canes in empty gasoline tins on clean wet sand. In March, when the canes were cut open, several were found to have a characteristic odor of pineapple and exhibited a mass of black spores that proved to be those of the pineapple disease, *Thielaviopsis ethaceticus* Went.

In a recent paper G. L. Fawcett[1] states, in referring to this finding of pineapple disease in the province: "An entomologist who was rearing and sending parasites of the sugar-cane borer to North America had placed some canes of the variety P. O. J. 2725 originating from one of the lots of this station (in cans) during three months under favorable conditions for the best growth of the disease; those which were examined were found to be heavily attacked by pineapple disease. All of these canes had been perforated with an instrument used previously in Jujuy for the same object; it was surely not sterilized before being used here . . . an experiment was repeated using the same cane and treatment. . . . On the first of May, 1930, three months later, they were examined and not one trace of pineapple disease was found. . . ."

This steel punch was made in Tucumán and never taken out of the Province. The writer made several trips to Jujuy, but no experiments were carried on there that required the use of such an instrument.

In October, 1929, some small pieces of cane then being used for rearing weevil larvae (*Acrotomopus atropunctellus* Boh.) showed signs of pineapple disease. This cane had been obtained from a field lying directly between Tafi Viejo and the experiment station at Tucumán, Argentina.

The writer then made another experiment: On October 28, he carefully sterilized in alcohol 3 jars and a cane knife and took them to the same field near Tafi Viejo. He cut short pieces of cane near the ground and placed 4 or 5 pieces in each jar. He then sealed the jars and took them back to the laboratory. On November 19 one jar showed development of black spores, and the next day another jar also showed signs of black spores. Later he opened the jars and filled them with a solution of formalin to preserve the canes. One of the jars showing the black spores was submitted to the Bureau of Plant Industry, United States Department of Agriculture,

[1] Fawcett, G. L. La putrefacción negra de la caña de azucar. Rev. Indus. y Agr. Tucumán 21: 55–59. 1931.

and the material was examined by Dr. George Sartoris, who determined it to be infected by *Thielaviopsis ethaceticus*, the cause of pineapple disease.

The methods employed in the sugar-cane-borer parasite work were such as to eliminate the possibility that the pineapple-disease infection could be brought into Tucumán on infected tools used in this work. The writer's observations and experiments have established definitely the presence of pineapple disease in the field in the Province of Tucumán, Argentina.—H. A. JAYNES, Associate Entomologist, Bureau of Entomology, U. S. Department of Agriculture.